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Gerardo Offimaria Ocfemia, 1891–1959 403

Healthy tip cuttings from some mosaic-diseased Asiatic chrysanthemums: some benefits and other effects of heat treatment ..... Philip Brierley and Paul Lorentz 404

Comparative incidence of graywall and internal browning of tomato and sources of resistance ..... Harry H. Murakishi 408

Yields of sour cherry affected by ring spot and yellows viruses ..... E. J. Klos and K. G. Parker 412

Host-parasite relations of Leptodiscus terrestris on alfalfa, red clover, and birdsfoot trefoil ..... Donald V. McVey and J. W. Gerdemann 416

Reversal of fungitoxicity of 8-quinolinol by amino acids and other chelators ..... George A. Zentmyer, Saul Rich, and James G. Horsfall 421

Factors affecting field spread of potato virus Y in south Florida ..... John N. Simons 424

Serological studies of commercially produced plant-virus antisera ..... Committee on Virus Type Culture Collection, American Phytopathological Society 428

Studies in the diseases of Mangifera indica. XI. The effect of boron on mango necrosis ..... S. N. Das-Gupta and C. Sen 431

Relation of nitrogen to disease development in rice seedlings infected with Helminthosporium oryzae ..... S. B. Chattopadhyay and J. G. Dickson 434

Induced mutation and cytological investigations in Helminthosporium oryzae ..... S. B. Chattopadhyay and J. G. Dickson 439

The histopathology of root-knot nematode infections in soybeans ..... Victor H. Dropkin and Paul E. Nelson 442

Relation of insects to internal cork of sweet potato in Louisiana ..... E. J. Kantack, W. J. Martin, and L. D. Newsom 447

Relation of microclimates to the development of powdery mildew of lettuce ..... W. C. Schnathorst 450

Genetic control of the host-parasite relationship in wheat powdery mildew ..... H. R. Powers, Jr., and W. J. Sando 454

Reduction in pathogenicity and toxin production in diseased Helminthosporium victoriae ..... G. D. Lindberg 457

Potato virus F latent in an imported variety and resistance to the virus in an interspecific potato hybrid ..... R. H. Bagnall 460

A necrotic pod streak of pepper caused by tobacco mosaic virus ..... Harry H. Murakishi 464

Studies on yellow bud mosaic virus ..... Harry P. Karle 466

PHYTOPATHOLOGICAL NOTES

Relations of variety, temperature, and seed immaturity to pre-emergence damping-off of castorbean ..... C. A. Thomas 473

A whole-mount technique for studying infected leaves ..... P. K. Isaac 474

Gamma radiation dose response of some decay pathogens ..... Louis Beraha, M. A. Smith, and V. R. Wright 474

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GERARDO OFFIMARIA OCFEMIA, 1891–1959

Victoria M. Ela and James G. Dickson

The death of Dr. Gerardo Offimaria Ocfemia at the age of 68 at Pasay City, Manila, Philippines, on November 17, 1959, abruptly ended a fine and useful career of service to plant pathology, mycology, virology, and agriculture. Through 42 years of devotion to teaching, research, and service, he richly deserved the title "Dean of Filipino Plant Pathologists."

Dr. Ocfemia was born at Guinobatan, Albay, Philippines, on April 23, 1891. He received the B.Sc. degree at the College of Agriculture, University of the Philippines, in 1915, and the M.S. in 1920. While serving as Instructor at the University of the Philippines he was awarded the University Fellowship for advanced study, in 1920, and received the Ph.D. from the University of Wisconsin in 1923. He returned to the Philippines and devoted his life to teaching, research, and service.

He served the College of Agriculture of the University of the Philippines from 1915 until his retirement, on April 23, 1957. He was assistant instructor in Agronomy, 1915–1917; assistant instructor in Plant Pathology, 1918–1920; instructor, 1920–1924; assistant professor, 1924–1932; associate professor, 1932–1945; and professor, 1945–1957. He was appointed acting head of the department in 1928, the first Filipino to serve in this capacity, and head in 1933, which position he held until the close of the academic year in 1955.

His retirement from active service as Professor of Plant Pathology was extended one year beyond the compulsory retirement date by executive order of Ramon Magsaysay, then President of the Philippines. Upon retirement he was asked to organize and head the Department of Plant Pathology, Institute of Agriculture, in the privately endowed Araneta University in Grace Park, Caloocan, Rizal, where he was serving actively at the time of his death.

Dr. Ocfemia's outstanding contributions as teacher and as investigator were widely recognized. The impact of his cheerful, dynamic, and devoted personality was equally effective on farmer, student, faculty, and administrator. His major research contributions were concerned with the use of precise experimental methods in the study of plant viruses. He reported on the host-range, vector-transmission, and control of Fiji disease and mosaic of sugarcane, bunchy-top and mosaic of abacá, cadang-cadang of coconut, and the transmission of the aspermy virus to *Canna indica*. He published 56 scientific and 38 popular papers, and assisted in the preparation of 59 scientific papers by his students. He was managing editor of the Philippine Agriculturist from 1938 to 1941 and from 1947 to 1955.

Dr. Ocfemia served on many national and international committees and conferences. He was the recipient of a John Guggenheim Memorial Foundation fellowship in 1955, and concurrently visiting Investigator in the Rockefeller Institute for Medical Research. He was one of 10 scientists awarded the Distinguished Service Medal and Diploma of Honor in the 1955 Science Celebration in the Philippines.

He was a member of local and foreign scientific and honorary societies. He was a charter member of the Society for the Advancement of Research and of the National Research Council of the Philippines, and Chairman of the Plant Pathology and Mycology Section. He was a member (fellow) of the American Association for the Advancement of Science, the American Phytopathological Society, the Botanical Society of America, the New York Academy of Sciences (Fellow 1959), Sigma Xi, Gamma Sigma Delta, and Phi Kappa Phi.

Dr. Ocfemia, on his return to the Philippines after graduate study at Wisconsin, was married to Epifania San Valentin, of Los Baños, Laguna. Five children were born into this happy family. He often stated that his only avocation was his family.

DR. GERARDO O. OCFEMIA
April 23, 1891–November 17, 1959

PHYTOPATHOLOGY for May (50:329-402) was issued May 17, 1960).

HEALTHY TIP CUTTINGS FROM SOME MOSAIC-DISEASED ASIATIC CHRYSANTHEMUMS; SOME BENEFITS AND OTHER EFFECTS OF HEAT TREATMENT

Philip Brierley and Paul Lorentz

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Accepted for publication December 9, 1959.

SUMMARY

Healthy tip cuttings were obtained from 19 of 22 heat-treated mosaic-infected chrysanthemums from Japan and 5 of 10 from Taiwan after 2–8 months at 35°C. Healthy plants tend to grow taller and have larger and earlier blooms of better lasting quality and stiffer stems than corresponding mosaic-affected plants. Such differences are sometimes striking but are often negligible because of high mosaic tolerance of some Asiatic varieties. Heating at 35° induced true fasciation in some and devernalization in some Japanese sorts, but neither effect appeared in the American or English varieties heated similarly.

As reported (1), 16% of the Japanese chrysanthemums (*C. morifolium* (Ramat.) Hemsl.) imported by John L. Creech were infected with tomato aspermy virus, and 14% with mosaic viruses. To salvage as many as possible of these imported chrysanthemums the plants infected with virus were heat-treated at about 35°C, a temperature previously found effective in producing virus-free tip cuttings of some chrysanthemum varieties (2, 3). These cured plants and a few others were compared with infected plants in tests of flower quality. Additional effects of heat treatment are described. Some mosaic-infected chrysanthemums from Taiwan were heated in the same manner for comparison.

Methods.—A greenhouse compartment described earlier (2) was used. In January, 1958, the thermostat was set for 35°C, but summer temperatures often exceeded this figure in the compartment. Equipment failures caused temperature drops, twice in February and twice in August, and an uncontrolled rise in temperature killed the remaining plants in February, 1959.

Vigorous plants, usually 2 of each variety in 6-in. pots, were subjected to heat. Tip cuttings were taken from these plants each month until virus-free tips were obtained or until the plants died. When leading shoots were killed by the heat, cuttings were taken from tips of lateral or stolon shoots. Cuttings about 1 cm long were rooted under mist and transferred to soil in pots. When a potted cutting was large enough to furnish a good scion, the scion was grafted on a mosaic-indicator variety that had proved suitable in earlier tests. Grafts were discarded if the indicator developed mosaic symptoms. Indicator varieties normally responded in 4–6 weeks, but when mosaic virus titer was reduced by heat the interval was occasionally prolonged to 3 months and once even to 5. To avoid confusing delayed response with true therapy, 6 months on the indicator variety was adopted as the requirement for accepted heat cure. The plants were fertilized monthly and sometimes shifted to larger pots to maintain vigorous growth for 6 months.

Plants infected only with tomato aspermy virus were treated in the same way up to the potted-cutting stage.

Table 1.—Healthy tip cuttings from 19 of 22 mosaic-affected Japanese chrysanthemums grown in pots at 35°C for 1–10 months.

P.I. no.	Virus-free cuttings over total tested for indicated period (months):									
	1	2	3	4	5	6	7	8	9	10
235615	0/4	0/4	0/10	0/6	2/10	3/7	1/2	2/4		
630	0/9	0/4	0/6	0/4	0/4	1/4	0/5	3/9	3/8	4/10
903	0/6	0/3	0/8	1/10	1/8	4/10	11/12	6/7		
907	0/3	2/6	0/8	1/7	0/4	4/5				
910	0/2	0/1	0/10	0/5	0/5			2/3	0/2	1/3
911	0/3	0/3	0/8	0/2	0/2	0/3		1/6	0/6	3/6
915	0/9	0/8	0/16	1/6	3/11	1/9	2/7	1/10		
917	0/5	0/3	0/6	0/4	3/6	2/5	4/4	1/3		
236029	0/8	2/8	0/12	4/7	7/7	5/6				
031	0/11	2/6	2/11	5/7	10/11					
032	0/4	0/6	0/12	0/5	0/7	1/7	4/8	2/6		
036	0/10	0/11	1/15	0/7	0/12	4/13				
038	0/6	0/9	0/17	0/5	0/10	1/11	8/17	0/4		
039	0/6	0/7	0/9	0/3	0/3					
048	0/6	0/9	0/12	0/7	1/6	3/6	3/5	1/6		
078	0/4	2/5	0/8	1/6	2/7	10/10				
125	0/6	0/5	3/8	1/3	2/8	3/3				
126	0/7	0/8	0/10	0/5	4/9	4/4	0/1	0/2		
128	0/4	0/3	0/9	0/5	0/7	3/4	1/3	1/2		
174	0/6	0/7	2/11	1/8	2/6	0/5	0/4			
179	0/6	0/8	0/10	0/3						
185	0/6	0/10	0/8	0/4						
Total	0/131	8/134	8/224	15/119	37/143	49/112	34/68	20/62	3/16	8/19

When the cutting had two full-grown leaves these were ground and used to inoculate 3 young tobacco plants. If no infection developed in the tobacco in 14 days, the test was repeated with 2 additional leaves and 3 additional tobacco plants. If this test was negative after 14 days, the cutting was judged free of tomato aspermy virus. Several plants carrying both tomato aspermy and a mosaic virus were treated as for mosaic alone. When tips free of mosaic virus were reclaimed the aspermy test was applied to these. Aspermy virus was consistently eliminated after 1–2 months' heating; this virus was never detected in cuttings freed of mosaic viruses.

Experimental results.—Table 1 summarizes the data on reclamation of healthy tips from 19 Japanese chrysanthemums. Normal cuttings were obtained from 4 varieties after heating for only 2 months, from 3 others after 3 months, 2 after 4 months, 4 after 5

Table 2.—Flowering performance of some Asiatic and American chrysanthemums before and after elimination of mosaic (M) or mosaic plus aspermy (TAV) virus.

Variety or P.I. no.	Cured or diseased	Short days started	Date of bloom	Height (cm)	Flower diam. (cm)	Disbudded	Flower class	Benefits[a]	Notes
		1958	1959						
Nightingale	Cured	Nov. 5	Jan. 5	72	20	Yes	Spider	TEL	
	TAV + M		Jan. 9	67	19				No distortion
Tuxedo[b]	Cured	Nov. 17	Jan. 19	60	9	No	Anemone	TEL	
	M		Jan. 21	55	8				
Bronze Daisy	Cured	Nov. 17	Jan. 5	41	7	No	Single	T	
	M		Jan. 5	36	7				
235907	Cured	Nov. 17	Jan. 29	57	24	Yes	Standard	TEL	
	M		Feb. 1	55	23				
236078	Cured	Nov. 17	Jan. 19	64	24	Yes	Standard	TEL	Stiffer stems
	M		Jan. 23	55	20				
236029	Cured	Nov. 17	Jan. 15	68	11	Yes	Anemone	TE	
	M		Jan. 19	62	11				
236031	Cured	Nov. 17	Jan. 13	40	11	Yes	Anemone	TE	
	TAV + M		Jan. 19	35	11				Slight twist
235903	Cured	Nov. 17	Jan. 12	51	23	Yes	Spider	TL	
	M		Jan. 12	48	22				
236048	Cured	Nov. 17	Jan. 12	48	12	Yes	Standard	TL	
	M		Jan. 12	37	10				
236128	Cured	Nov. 17	Jan. 14	44	14	Yes	Standard	TEL	
	M		Jan. 19	37	12				
236126	Cured	Nov. 17	Jan. 19	34	13	Yes	Standard	L	
	M		Jan. 19	36	11				
235915	Cured	Nov. 18	Jan. 19	71	27	Yes	Spider	L	
	M		Jan. 19	72	25				
235917	Cured	Nov. 18	Jan. 5	44	19	Yes	Cascade	None	Better not disbudded
	M		Jan. 5	47	20				
235615	Cured	Dec. 15	Feb. 9	42	10	Yes	Pompon	None	Better not disbudded
	M		Feb. 9	41	10				
Detroit News[c]	Cured	Dec. 15	Feb. 11	52	12	Yes	Standard	TEL	
	M		Feb. 19	38	9				
		1959							
236125	Cured	Jan. 16	Mar. 11	46	15	Yes	Standard	TEL	Stiffer stems
	M		Mar. 15	43	14				
236038	Cured	Feb. 19	Apr. 17	41	11	No	Spoon	TE	
	M		Apr. 23	38	11				Slightly irregular
Nightingale	Cured	Mar. 6	May 12	57	22	Yes	Spider	TEL	
	TAV + M		May 18	52	20				No distortion
236032	Cured	Apr. 14	June 16	62	8	No	Spoon	TL	
	M		June 16	54	7				
Taiwan No. 24	Cured	Apr. 15	June 18	65	17	Yes	Standard	TEL	
	M		June 22	55	15				
236174	Cured	May 12	July 15	83	9	No	Standard	T	Better when disbudded
	TAV + M		July 15	79	9				
235910	Cured	June 8	Aug. 14	85	12	No	Standard	EL	Better when disbudded
	M		Aug. 19	85	11				
235911	Cured	June 8	Aug. 31	113	17	No	Spider	EL	Better when disbudded
	M		Sept. 5	120	15				
235630	Cured	June 22	Aug. 7	47	7	No	Pompon	E	
	M		Aug. 10	47	7				
Taiwan No. 26	Cured	Aug. 4	Oct. 19	84	14	No	Spider	T	
	M		Oct. 19	80	14				

[a] T = plants taller, E = flowers earlier, L = flowers larger.
[b] Reported as Yellow Doty in (2).
[c] Reported as Good News in (2).

months, 4 after 6 months, and 2 after 8 months. The proportion of healthy cuttings tended to increase as length of heat treatment increased, but rarely did all shoots prove mosaic-free even after many months of heating, and no mosaic-affected plant was completely freed of virus.

The mosaic viruses of the Japanese selections varied widely in virulence as judged by their reactions on our indicator varieties, but we noted no correlation between virulence and difficulty of reclaiming healthy tissues from its host. Variety seems to influence ease of therapy, for varieties infected with mild mosaic

Fig. 1.—A) Heat-cured (left) and mosaic-affected plants of P.I. 236078, Togamano-Kumo. The cured plant bloomed January 19, the mosaic-affected plant January 23. Photographed January 29, 1959. **B)** Heat-cured (left) and mosaic-affected plants of P.I. 236029, Hakusa. The cured plant bloomed January 15, and the mosaic-affected one January 19. Photographed January 29, 1959. **C)** Fasciation in scion of P.I. 235903, Oukounobikari, from a cutting taken after 6 months at 35°C. **D)** Devernalized and normal cuttings of P.I. 236164, Hana Chidoi, from plants heated 15 weeks at 35°. Some leaves were removed from the devernalized plant (left) to show the short internodes more clearly.

viruses occur in both the 2-month and the 8-month therapy classes. Some varieties are damaged early and soon killed by the heat treatment. Leaves are sometimes bleached to yellow or to white, and shoots may be killed nearly to the soil level, after which basal or stolon shoots may appear to replace them.

Of the Taiwan chrysanthemums, three were freed of mosaic in 2 months, one in 5 months, and one in 6 months, while five others failed to produce a virus-free tip after 4–9 months' heating. There is little evidence that Taiwan viruses resist heating more than Japanese viruses do, or that varieties from Taiwan are more tolerant to heat.

Some benefits of heat therapy.—Several of the heat-cured Japanese varieties, some of those from Taiwan, and 4 American varieties previously reported cured were compared with the corresponding mosaic-diseased stocks in flowering tests. Cuttings of the same age were placed on benches without supplementary light in winter, or in summer were shaded with black cloth to provide an 8-hour day.

A few varieties, such as those shown in Fig. 1-A,B, were strikingly improved when freed of mosaic viruses. Healthy plants tend to grow taller and to produce larger, earlier flowers than the corresponding mosaic-diseased plants. In some instances stem stiffness and lasting quality also improved (Fig. 1-A,B). Such differences, however, were commonly slight and sometimes not even demonstrable in small-scale comparisons (Table 2). No correlation is evident between the virulence of a mosaic virus and the improvement attained by eliminating it. The two varieties in Fig. 1-A,B showed marked improvement on the elimination of mild mosaic viruses. In contrast, varieties infected with more virulent mosaic viruses (P.I. 236031, 236032, 236038, and 236126, Table 2) showed less improvement. A virulent mosaic virus in an intolerant chrysanthemum variety is very damaging (e.g., Detroit News, Table 2), but the Japanese chrysanthemums were all horticulturally acceptable and thus would not be expected to include the more damaging virus-variety combinations.

Fasciation and devernalization in heated chrysanthemums.—Several of the Japanese chrysanthemums developed true fasciation (Fig. 1-C), and some were typically devernalized (Fig. 1-D) after heating. Neither effect was noted in the corresponding stock plants held at greenhouse temperatures (18–23°C in winter, higher in summer) or during their stay at 35°. Both effects became evident in the rooted tip cuttings after transplanting to soil, at the stage when normal cuttings were ready to graft to the indicator variety. The flattened stems of fasciated plants and the many close-set nodes of those devernalized were inconvenient for grafting, but when used as scions such stems readily united with the understocks and soon reverted to normal growth. Fasciation appeared chiefly in cuttings from plants heated 6 months or longer, and was only temporary.

Many garden chrysanthemums, including Japanese garden varieties, are devernalized if wintered in a greenhouse at 18–23°. Normal shoot growth in these is resumed if the plants are allowed a cool period or treated with gibberellin. The group of varieties discussed herein are florists' types that show no tendency to devernalize at 18–23°, but often do so after several months at 35°. These soon resume normal shoot growth when grafted on Good News stocks. If not grafted but merely held at 18–23° they resume normal shoot growth during the spring.

The variety Hana Chidoi (P.I. 236164) carried tomato aspermy virus and a mosaic virus. These viruses were readily eliminated, but since both fasciation and devernalization appeared in this variety, propagations were continued up to 7½ months to provide data (Table 3) on the prevalence of these defects in heated stocks. Fasciated plants appeared in low frequencies only after 6 months' heating. Devernalized plants were most common in cuttings taken in October, November, and January after 3½ to 7 months' heating, at which times they outnumbered the normal plants. At no time were all cuttings exposed to 35° devernalized.

Discussion.—The chrysanthemum mosaics and aspermy seem likely to be of Asiatic origin, for these diseases are prevalent in chrysanthemums imported directly from Japan and Taiwan. Moreover, many Asiatic chrysanthemums carrying the causal viruses have mild symptoms or none, thus showing the adjustment to disease characteristic of long association. The rosette viruses, which were absent from our sampling of chrysanthemums from Asia though common in European sorts (2), are presumably of European origin. Several English varieties carry rosette viruses without symptoms, suggesting long association and adaptation. Chrysanthemum stunt, flower distortion, and aster yellows are evidently native to North America, although no varieties are known to show marked tolerance to these diseases. Possibly these diseases have a long history here in some other hosts, but a relatively short history in chrysanthemum.

Heat therapy of virus-diseased plants was first demonstrated 35 years ago, when sugar cane was freed of sereh disease by hot-water treatment. Many successes followed, some with hot-water and some with hot-air treatments, notably in sugar cane, stone fruits, strawberries, Irish potatoes, and several other plants infected with yellows-type viruses. Kassanis (7) made

Table 3.—Fasciation and devernalization in Hana Chidoi (P.I. 236164) chrysanthemum exposed to 35°C for 3½–7½ months beginning June 23, 1958.

Duration of treatment (months)	Plants			
	Treated	Fasciated	Devernalized	Normal
3½	5	0	4	1
5	10	0	6	4
6	6	1	1	4
7	11	2	8	1
7½	5	1	1	3

a notable advance when he demonstrated that some common aphid-borne viruses, such as those of cucumber mosaic and tomato aspermy, can be heat-inactivated. He found that several spherical viruses are readily curable; some host plants are completely freed of them by short periods of heating. Kassanis (7) suggested that virus synthesis is stopped but degradation continues at higher temperatures. The rod-shaped viruses he tested were not cured, but their titer was lowered by heat treatment.

Hollings and Kassanis (3) reported cure of stunt-infected as well as aspermy-infected chrysanthemums. These English workers use a small cabinet, accurately controlled at 36°C, and relatively short intervals, such as 21–32 days. Our heat chamber, though less accurately controlled, is open to natural daylight, apparently a marked advantage. Some chrysanthemum varieties have withstood 35° for a full year, in contrast to the experience of Hollings and Kassanis (3), who said that "chrysanthemum plants can be heated only in summer, and even then some usually die."

Holmes (4, 5, 6) reclaimed virus-free tips from dahlia infected with tomato-spotted-wilt virus, from sweet potato infected with foliage-spotting virus, and from Nightingale chrysanthemum infected with tomato-aspermy virus. He used no heat treatment but selected short cuttings or scions from plants he judged not fully invaded by the respective viruses. Many viruses lag behind the growing shoot tips of their hosts. Some lag enough that short cuttings or scions are virus-free. Others lag only at higher temperatures that depress virus titer, and chance offers an occasional virus-free tip. Higher temperatures, longer exposures, and larger numbers of trials tend to increase the chance of success, which is not as assured in the stubborn viruses as it is with viruses more readily inactivated.—U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

LITERATURE CITED

1. Brierley, P. 1958. Tomato aspermy in chrysanthemums from Asia and Europe. Plant Disease Reptr. 42:61-62.
2. Brierley, P., and F. F. Smith. 1958. Some characteristics of eight mosaic and two rosette viruses of chrysanthemum. Plant Disease Reptr. 42: 752-763.
3. Hollings, M., and B. Kassanis. 1957. The cure of chrysanthemums from some virus diseases by heat. J. Roy. Hort. Soc. 82:339-342.
4. Holmes, F. O. 1955. Elimination of spotted wilt from dahlias by propagation of tip cuttings. Phytopathology 45:224-226.
5. Holmes, F. O. 1956. Elimination of foliage spotting from sweetpotato. Phytopathology 46: 502-504.
6. Holmes, F. O. 1956. Elimination of aspermy virus from the Nightingale chrysanthemum. Phytopathology 46:599-600.
7. Kassanis, B. 1954. Heat therapy of virus-infected plants. Ann. Appl. Biol. 41:470-474.

COMPARATIVE INCIDENCE OF GRAYWALL AND INTERNAL BROWNING OF TOMATO AND SOURCES OF RESISTANCE

Harry H. Murakishi

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The isolate of TMV used was kindly furnished by Dr. J. S. Boyle, Pennsylvania State University.

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SUMMARY

Graywall (GW) and internal browning (IB) occurred together in fields in Michigan in 1958 and caused severe losses. Studies in which both disorders were reproduced simultaneously confirmed that GW and IB have nearly identical symptoms. GW was induced by low light intensity in TMV-free plants, whereas IB was caused by late infection with a strain of tobacco mosaic virus (TMV). Low light also increased the incidence of IB in susceptible varieties. Two GW-resistant varieties, Indian River and Manalucie, were susceptible to IB but Holmes' and Walter's TMV-resistant breeding lines were resistant to both GW and IB. Assays showed that IB reaction was correlated to TMV reaction. A tobacco-etch-resistant line proved susceptible to IB.

Two diseases, both affecting the fruit of tomato (*Lycopersicon esculentum* Mill.), have strikingly similar symptoms. One of the disorders, known as internal browning (IB), has been demonstrated by Boyle and Wharton (2) to be the result of late infection of tomato plants by certain severe strains of tobacco mosaic virus (TMV). They attributed the necrotic symptoms to shock from virus invasion and accumulation in the fruit, followed by a hypersensitive response of the host. The second disorder, commonly referred to as graywall, is designated as GW herein. It was first described by Conover (3) as vascular browning and is believed to be independent of TMV infection as shown by Stoner and Hogan (14). The cause of GW has not been found. The disorder differs from IB in that fruit symptoms were induced by any one of several nonparasitic factors such as low light intensity (6, 10), low temperature (6), high soil moisture (6, 16) and soil compaction (6).

Resistance to GW has been reported by Hayslip et al (7). They found Indian River and Manalucie to be resistant and Homestead and Rutgers susceptible to GW. Resistance to presumably the same disorder was reported by Jones (10) in Strain A Globe tomato. Resistance to IB has not been found in commercial varieties but Holmes (8) observed that a TMV-re-

sistant line was free of IB in a field where adjacent rows of Valiant were naturally infected with mosaic and 35% of the fruit had IB. Walter (17, 18) reported resistance to TMV and tobacco etch virus (TEV) in tomato breeding stocks but made no mention of their reaction to either GW or IB.

In 1958, incidence of a fruit disorder similar to GW and IB was high in Michigan, chiefly in the leading market variety, Fireball. Severely affected fruit from several farms were tested for TMV. Assays on sensitive local-lesion hosts in about half of the samples were either negative or yielded only 1–2 lesions, whereas the remaining samples induced abundant TMV lesions. In light of the statement of Boyle and Wharton (2) that virus accumulates in IB fruit, the findings were interpreted to mean that both GW and IB were present in the same fields. To test that assumption, an experiment was made to determine the reaction of Fireball simultaneously to low light intensity, a factor known to induce GW, and to late infection with TMV. Subsequently, varieties with known reactions to GW and breeding lines with known resistance to TMV were tested in the field to determine their response to the 2 disorders. Preliminary results have been reported (12).

Materials and methods.—Experiments were performed in the greenhouse and field. Seeds, if recently harvested, were soaked 15 minutes in 10% trisodium phosphate (9), dried, and sown in steamed vermiculite. For the greenhouse test, seedlings were transplanted to steamed U.C. (1) soil mix, 1:1 sand and peat, in 10-in. pots. A soluble fertilizer of analysis 12-31-14 was added weekly soon after transplant. The experiment was conducted during April to mid-June. The varieties tested were Fireball (Lot 861, Harris Seed Company) and Homestead 24 (Lot 344.11, Indian River Field Laboratory, Fort Pierce, Florida). Twenty plants of each variety were randomized within each of the following treatments: TMV-free–low-light, TMV-free–high-light, TMV-inoculated–low-light, and TMV-inoculated–high-light. All plants in the 4 series were indexed a few days before treatments were started. A young leaf was removed from a side shoot nearest the first cluster of fruit and rubbed face to face on a carborundum-dusted leaf of *Nicotiana tabacum* var Xanthi-nc, a sensitive local-lesion host for TMV (14) and a systemic host for cucumber mosaic virus and for TEV. The leaf-to-leaf method of virus transfer (unpublished data) was most convenient, and as reliable as the conventional juice–leaf-rub method. Soon after the fruits were harvested, all uninoculated plants were indexed a second time. When an affected fruit was found on an uninoculated plant, the fruit as well as a fresh leaf sample was indexed again, this time with the juice–leaf-rub method. However, in every case where affected fruit appeared in the uninoculated series, no virus was ever isolated. All TMV-inoculated tomato plants indexed by the leaf-to-leaf method yielded abundant local lesions on Xanthi. It is therefore appropriate to designate uninoculated plants as TMV-free and TMV-inoculated plants as TMV-infected. In the field, procedures were similar except that the second indexing at the end of the test was confined to the uninoculated plants with affected fruit. In contrast to the greenhouse test, uninoculated plants in the field could be distinguished from susceptible TMV-inoculated plants, which displayed a yellow mottle at the end of the experiment. Again, no virus was ever isolated from uninoculated plants or fruit.

In the greenhouse, when fruits were two-thirds mature, plants in the low-light series were covered with a mesh-fabric shade (Saran mesh fabric, Chicopee Manufacturing Corp., Cornelia, Georgia) designed to cut out about half the light. The fabric was arranged on a light wooden framework so that the plants were shaded on the top and all sides down to pot level. In the high-light series the plants were left uncovered. Light, measured with a Weston Illumination Meter on cloudy and sunny days, ranged from 700 to 4000 ft-c in the low-light series and from 1400 to 8500 ft-c under high light. Average temperature was 77.8°F (average range, 68.4–88.1°) under high light, and 1–2° lower beneath the shading fabric.

The virus used was a strain of TMV furnished by Dr. J. S. Boyle, who had isolated it from an IB fruit. In subsequent tests, he found it would induce IB. The virus was increased and maintained in Turkish or Havana 38 tobacco. In inoculations of tomato plants, a 1:25 dilution of crude juice to which carborundum powder was added was applied with a brush along the main stem above and below the first cluster of fruit as it approached maturity. The plants at this stage were about 4 ft. tall and had 2–4 clusters of fruit. All fruits were harvested on an individual plant basis about 20–22 days after inoculation.

Two experiments were conducted during late June to the end of August in the Botany and Plant Pathology field plots at East Lansing. Within the past 10 years, no tomatoes had been grown in the test area. Also tested besides the Fireball and Homestead varieties were Manalucie, 3 Walter's TMV-resistant breeding lines, a Holmes' TMV-resistant line, and a TEV-resistant Walter's breeding line. In the first field test, each variety or line was grown in replicated paired 5-plant rows. One row, representing the low-light series, was covered with a 3-ft-wide shading fabric of the type previously described. The other row of the pair represented the high-light series. Light readings on cloudy and sunny days ranged from 1200 to 5000 ft-c under the shade and from 3500 to 10,000 ft-c in the unshaded plots. Respective average air temperatures near the center of the plants were 78.8 and 79.9°F, with respective average ranges of 66.4–94.0 and 66.4–95.8°F.

A third test was conducted simultaneously with the second in an adjacent field, but under natural light without shading. The temperatures were the same as those in the high-light series of the previous experiment.

Table 1.—Effect of light intensity and TMV on incidence and severity of graywall (GW) and internal browning (IB) in the greenhouse.

		Virus-free plants[a]				TMV-infected plants[a]			
Variety	Light intensity	GW plants (%)	Total fruit	GW fruit (%)	Av. severity[b]	IB plants (%)	Total fruit	IB fruit (%)	Av. severity[b]
Fireball	Low	10.0	148	2.7	3.3	60.0	183	13.7	2.5
	High	10.0	201	2.5	2.0	30.0	205	8.3	2.4
Homestead	Low	25.0	121	6.6	3.5	100.0	136	55.9	3.2
	High	5.0	159	1.3	2.5	60.0	127	21.3	3.1

[a] Twenty plants of each variety were used for each of the 4 treatments.
[b] Based on Boyle's severity scale of 1.0–5.0 (av. of all affected fruit).

Results.—During the investigation, it became evident that distinguishing GW from IB by symptoms was not possible with any degree of certainty. Some differences were noted, such as the "pinched shoulder effect" (6) in IB fruit. However, IB fruit on the same plant often lacked this external symptom. Furthermore, the internal symptoms of GW and IB were similar to those described for both disorders (4, 13, 19). Therefore, differentiation was based not on symptoms but on whether the affected fruit was produced on TMV-infected plants or on TMV-free plants. GW and IB occurred in both Fireball and Homestead in the greenhouse experiment (Table 1). In TMV-free plants under low light intensity, 2 of the 20 Fireball plants and 5 of the 20 Homestead plants produced GW fruit. Under high light, 2 of the 20 Fireball and 1 of the 20 Homestead plants produced GW fruit. Fireball and Homestead respectively had 2.7 and 6.6% GW fruit in the TMV-free–low-light series and 2.5 and 1.3% GW fruit in the TMV-free–high-light treatment. Severity of GW symptoms was greater under low light than under high light. The combination of TMV and low light induced 13.7% IB fruit in Fireball and 55.9% IB fruit in Homestead. In the TMV–high-light series, Fireball plants had 8.3% IB fruit and Homestead had 21.3% IB fruit. The results thus indicate that these 2 varieties are susceptible to both GW and IB but that TMV exerted a greated influence on IB formation than did low light intensity on GW.

In the second experiment (Table 2), in the field, a high proportion of the susceptible Fireball and Homestead plants in the TMV-free–low-light series produced GW fruit. Eight of the 10 Fireball plants and 5 of the 10 Homestead plants had affected fruit. In the TMV-free–high-light series, 4 of the 10 Fireball and 1 of the 10 Homestead plants had GW fruit. Again, indexing to Xanthi showed no virus in either the foliar tissue or the affected fruit. The Indian River and Manalucie varieties, reportedly resistant to GW (7), showed no GW in the TMV-free–low-light series, and only 0.4% GW in the TMV-free–high-light series. P.I. 235673, W-1, and W-2 were also relatively free of GW in the TMV-free–high- and –low-light treatments.

Comparison of TMV-free and TMV-infected plants

Table 2.—The occurrence of graywall (GW) and internal browning (IB) in tomato varieties and breeding lines under high and low light in the field.

		TMV-free plants[a]			TMV-inoculated plants[a]		
Variety	Light intensity	GW plants %	Total fruit	GW fruit %	IB plants %	Total fruit	IB fruit %
Fireball	Low	80.0	327	15.0	90.0	275	29.1
	High	40.0	279	6.1	80.0	300	17.7
Homestead	Low	50.0	196	4.1	80.0	269	50.6
	High	10.0	243	0.4	100.0	383	56.7
Indian River	Low	0.0	303	0.0	100.0	346	29.2
	High	10.0	277	0.4	100.0	400	31.5
Manalucie	Low	0.0	220	0.0	100.0	266	30.8
	High	10.0	273	0.4	100.0	347	27.1
P.I. 235673	Low	10.0	145	0.7	10.0	192	1.6
	High	0.0	148	0.0	10.0	139	0.7
W-1[b]	Low	0.0	79	0.0	20.0	97	1.0
	High	0.0	164	0.0	20.0	87	5.8
W-2[c]	Low	0.0	72	0.0	0.0	84	0.0
	High	0.0	123	0.0	0.0	92	0.0

[a] Ten plants of each variety were used for each of the 4 treatments.
[b] Refers to Walter's STEP 305, advanced TMV-resistant line.
[c] Refers to Walter's 7-4-3-1-CH2-BK ACEMStW, combined resistances to TMV and TEV. In b and c, 5 plants were used for each treatment.

(Table 2), showed that TMV had a much greater influence on IB production than did low light intensity on GW. In the TMV-free–low-light series, 15.0% of the Fireball fruit were affected by GW, as compared to 29.1% IB fruit for the TMV-infected plants. Compared in the same treatments, Homestead had 4.1% GW and 50.6% IB fruit. Under high light conditions, similar differences were observed between TMV-free and TMV-infected plants of Fireball and Homestead. Indian River and Manalucie, the 2 varieties resistant to GW, proved susceptible to IB. TMV-infected Indian River had 29.2% IB under low light and 31.5% IB under high light. Similarly, TMV-infected Manalucie plants produced 30.8% IB fruit under low light and 27.1% IB under high light. TMV-resistant tomatoes had a very low incidence of both GW and IB. TMV-inoculated Holmes P.I. 235673 had 1.6% IB under low light and 0.7% IB under high light, whereas W-1 had 1.0 and 5.8% IB fruit, respectively. TMV-inoculated W-2 plants were free of IB, as well as GW under both low and high light.

Since resistance to IB appeared correlated with resistance to TMV, assays were made for TMV concentration. A 1-g composite sample of leaf tissue was obtained soon after harvest from each of 5 plants of each variety and breeding line under low and high light intensities. Each sample was homogenized in 10 cc distilled H_2O and the extract rubbed over 2 half-leaves of young Xanthi plants with a poster brush (15). Separate brushes were used for each variety in each treatment. Between samples, each brush was cleaned, rinsed in H_2O, and blotted dry. Assays of TMV-susceptible varieties Fireball, Homestead, Indian River, and Manalucie respectively produced an average of 62, 69, 115, and 67 lesions per half-leaf in the low-light series. Under high light, the corresponding average numbers of lesions were 59, 30, 98, and 88. Assays of the TMV-resistant lines P.I. 235673, W-1, and W-2 respectively produced an average of 11.5, 1.0, and 0.2 lesions per half-leaf. Under high light, the corresponding average numbers of lesions were 0.2, 6.0, and 0.0.

In the third experiment, conducted in the field under natural light without shading, the results generally agree with those of the comparable high-light series of the previous test. GW occurred only in the 2 susceptible varieties Fireball and Homestead (Table 3). Ten of 20 Fireball and 1 of the 20 Homestead plants produced GW fruit. None of the other varieties or breeding lines were found to have GW. Of the TMV-inoculated plants, all 20 Fireball and all 20 Homestead plants had IB fruits. IB fruits were also produced by 19 of 20 Manalucie plants inoculated with TMV, 9 of 20 Indian River, and 12 of 20 P.I. 18369 plants. The last is a breeding line that Walter (18) found resistant to TEV but not to TMV. The TMV-resistant lines P.I. 235673 and W-3 were completely free of IB. Assays of the TMV-inoculated breeding lines P.I. 23567, W-3, and P.I. 18369 respectively produced 0.2, 0.0, and 146.0 lesions per half-leaf of Xanthi.

Discussion.—One of the puzzling features of GW and IB has been their pronounced seasonal occurrence. Workers in the United States (5), England (20), and New Zealand (11) have reported that disorders of this type have been severe in certain years and unimportant in others. Climatic factors were suspected, and low light intensity (6, 10) and low temperature (6) have been shown to increase the incidence of GW greatly. On the other hand, Boyle and Wharton (2) found that the incidence of IB remained fairly stable over a 4-year span at Carversville, Pennsylvania, where the disease was considered endemic. Our greenhouse test, however, showed that TMV induced more IB under low light than under high light. This was confirmed in the field with Fireball variety. The seasonal variation in the incidence of these 2 disorders can possibly be explained on the basis of both climatic factors and TMV. Not only does low light intensity as from cloudy weather favor GW, as shown in this study, but low light in conjunction with late TMV infection favors IB. The severity of IB is accentuated when the varieties are extremely susceptible to both disorders.

GW and IB together increase the difficulty of control. Although the GW-resistant varieties Indian River and Manalucie were resistant to GW in the field tests, they were susceptible to TMV and to IB. The freedom from virus contamination in our greenhouse and field tests, however, would indicate that if fields are new or lack a reservoir of TMV in weeds and if care is exercised in preventing accidental infection, relative freedom from IB and GW in these 2 varieties is not unreasonable to expect. Where crops must be grown on the same field year after year, as is often the case, or where severe strains of TMV are present in weed reservoirs, as pointed out by Holmes (8), the need for combined resistances to both GW and IB is probably justified. Our results have shown that Holmes' TMV-resistant P.I. 235673 and Walter's TMV-resistant lines are likely sources of resistance for use in a breeding program for resistance to both disorders.—Department

Table 3.—The occurrence of graywall (GW) and internal browning (IB) in varieties and breeding lines under natural light in the field.

Variety or Breeding line	TMV-free plants[a]			TMV-inoculated plants[a]		
	GW plants (%)	Total fruit	GW fruit (%)	IB plants (%)	Total fruit	IB fruit (%)
Fireball	50.0	591	3.4	100.0	711	33.1
Homestead	5.0	431	0.2	100.0	592	53.2
Indian River	0.0	344	0.0	45.0	333	6.6
Manalucie	0.0	323	0.0	95.0	507	33.7
P.I. 235673	0.0	293	0.0	0.0	376	0.0
W-3[b]	0.0	164	0.0	0.0	161	0.0
P.I. 18369[c]	0.0	153	0.0	60.0	139	19.4

[a] Twenty plants of each variety were used for each treatment.

[b] Walter's 188-3-2-2(b)-1-1BkM, semi primitive TMV-resistant line.

[c] Breeding stock that Dr. Walter found was resistant to TEV but not to TMV. In b and c, 10 plants were used for each treatment.

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LITERATURE CITED

1. Baker, K. F. 1957. The U.C. system for producing healthy container-grown plants. Univ. Calif. Div. Agr. Sci. Manual. 332 p.
2. Boyle, J. S., and D. C. Wharton. 1957. The experimental reproduction of tomato internal browning by inoculation with strains of tobacco mosaic virus. Phytopathology 47:199-207.
3. Conover, R. A. 1949. Vascular browning in Dade County, Florida greenwrap tomatoes. Plant Disease Reptr. 65:353-356.
4. Gigante, R. 1954. La maturazzione a chiazzi dei fruitti di pomodoro. Boll. staz. patol. vegetale Roma 12:127-136.
5. Haenseler, C. M. 1949. Internal browning of tomatoes in New Jersey. Plant Disease Reptr. 37:336-337.
6. Hall, C. B., and R. A. Dennison. 1955. Environmental factors influencing vascular browning of tomato fruits. Proc. Am. Soc. Hort. Sci. 65:353-356.
7. Hayslip, N. C., J. M. Walter, and D. G. A. Kelbert. 1958. Indian River, a new disease-resistant tomato of general adaptability. Florida Univ. Agr. Expt. Stas. Gainesville Circ. S-111. 11 p.
8. Holmes, F. O. 1950. Internal-browning disease of tomato caused by strains of tobacco-mosaic virus from Plantago. Phytopathology 40:487-492.
9. John, C. A., and C. Sova. 1955. Incidence of tobacco mosaic virus on tomato seed. Phytopathology 45:636-637.
10. Jones, J. P. 1958. The relation of certain environmental factors, tobacco mosaic virus strains and sugar concentration to the blotchy ripening disease of tomato and the inheritance of the tendency to the disease. Ph.D. thesis, Ohio State Univ., 76 p. (unpublished).
11. Kidson, E. B., and D. J. Stanton. 1953. "Cloud" or vascular browning in tomatoes. I. Conditions affecting the incidence of "cloud." New Zealand and J. Sci. Technol. A 34:521-530.
12. Murakishi, H. H. 1959. Varietal response to factors influencing the expression of tomato internal browning symptoms. (Abstr.) Phytopathology 49:546.
13. Seaton, H. L., and G. F. Gray. 1936. Histological study of tissues from glasshouse tomatoes affected by blotchy ripening. J. Agr. Research 52:217-224.
14. Stoner, W. N. and W. D. Hogan. 1950. A report of graywall or internal browning of tomato in South Florida. Plant Disease Reptr. 34:379-380.
15. Takahashi, W. N. 1956. Increasing the sensitivity of the local-lesion method of virus assay. Phytopathology 46:654-656.
16. Taylor, G. A., C. B. Smith, and R. F. Fletcher. 1958. Influence of some environmental and nutritional factors on incidence of internal browning of tomato. Penna. State Univ. Agr. Expt. Sta. Bull. 629. 21 p.
17. Walter, J. M. 1956. Hereditary resistance to tobacco-mosaic virus in tomato. Phytopathology 46: 513-516.
18. Walter, J. M. 1956. Combination of resistances to tobacco-etch and tobacco-mosaic viruses in tomato breeding stocks. Phytopathology 46:517-519.
19. Wharton, D. C., and J. S. Boyle. 1957. The pathological histology of the internal browning disease of the tomato. Phytopathology 47:208-212.
20. White, H. L. 1938. Further observations of the incidence of blotchy ripening of the tomato. Ann. Appl. Biol. 25:544-557.

YIELDS OF SOUR CHERRY AFFECTED BY RING SPOT AND YELLOWS VIRUSES

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SUMMARY

Ring spot virus spread comparatively rapidly in a New York orchard, without definite pattern. Yellows symptoms were usually preceded by ring spot symptoms. Yellows virus spread more slowly than ring spot virus, with some apparent tendency to uneven distribution within the orchard.

In the year of first appearance of ring spot symptoms, trees with such symptoms yielded less than did healthy trees. In one case, yield was equal to that on healthy trees the following year; in another case, yield was not equal to that on healthy trees until the second year after first appearance of symptoms.

Yield of yellows-affected trees was lower than that of yellows-free trees in years succeeding the first symptom year. Trees with yellows had longer terminals and larger fruit than trees free of yellows.

During the observation period, the ring spot, yellows, and healthy trees groups did not differ in trunk circumference growth or total-solids content of the fruit.

Sour cherry yellows is considered the most important virus disease of the sour cherry, and ring spot is considered the most common. Precise information is needed on the extent of damage by the viruses as well as on their progress as they invade the planting. In particular, rate and manner of spread must be known if control measures are to be devised.

It is known that temperature has a profound effect on the expression of yellows symptoms (3, 6, 8) and also on ring spot symptom development. That and other factors may cause differences in disease effects in different areas. Reports have been made on influences on yield by Keitt and Clayton (2) Moore (7), and Lewis (5) and on the spread of one or both viruses by Keitt and Clayton (2), Lewis, (4), and Willison et al (9).

A study was undertaken in a single planting to determine the influence of these viruses on the growth and yield of maturing trees in the orchard. To do this it was necessary to study spread of the viruses within the orchard and to determine when each tree became infected.

Orchard spread of viruses.—In 1945, the junior author and Dr. W. D. Mills began a survey of a Montmorency orchard on the Horn Farm, at Sodus, N. Y.,

Table 1.—Spread of ring spot (RS) and yellows (Y) viruses in a 139-tree Montmorency cherry orchard (Horn Farm, Sodus, N. Y.), as indicated by the numbers of trees showing symptoms for the first time each year.

Year	No. trees showing first symptoms in year indicated			No. of trees with no symptoms in indicated year or before	
	RS only	RS and Y	Y only	Indexed + in 1950[a]	Indexed — in 1950[a]
1945	0	0	5[c]	46	88
1949[b]	3	0	0	43	88
1950	15	1	5	24	86
1951	36	6	8	11	49
1952	29	9	0	7	15[d]
1953	6	0	0	6	10[e]

[a] Results of index tests made in 1950 and in 1952 are taken into account. Note that no indexing was done before 1950.

[b] No additional trees with symptoms were found in surveys made in 1946 and 1947. No survey was made in 1948.

[c] These trees were presumably diseased when planted in 1940.

[d] Eight of these indexed as positive in 1952.

[e] Seven of these indexed as positive in 1952.

to determine the spread and behavior of yellows and ring spot viruses of sour cherry. The orchard, within an intensive cherry-growing area where other tree fruits are also grown, is bounded on the west by a cherry orchard a few years older, on the north by an apple orchard, on the east by a peach orchard, and on the south by an open abandoned field with a few wild cherry (*P. serotina* Ehrh.) trees in the hedgerow. These surveys were carried out in each year except 1948 in the period 1945–53. The orchard had been planted with trees on Mazzard rootstock in the fall of 1940.

In 1950, the authors began a study of the growth and fruiting habit of the trees in this orchard. All trees remaining at that time were indexed on peach seedlings. Four bud sticks were cut at random from each tree, 1 stick from each of 4 sectors of the tree. Four buds, 1 from each stick, were inserted in 2 Lovell peach seedlings, 2 buds on each seedling.

In 1945, 5 trees showed yellows symptoms (Table 1), supposedly because these trees had been propagated from infected buds. No other trees in the orchard showed symptoms of any kind until 1949. In the part of the orchard studied in detail are 139 of the original trees.

With one exception (among 50) yellows symptoms were not observed until after ring spot symptoms in the trees that indexed negative in 1950 (Table 2); in the one exception, the tree showed a trace of yellows in 1951, followed in 1952 by typical shock ring spot along with stronger symptoms of yellows. In some cases, yellows followed ring spot in a single season; more frequently, yellows followed ring spot by a year or more. On the other hand, of 26 trees that indexed positive in 1950 and showed yellows for the first time that year or later, 9 failed to show ring spot and 3 did so in years after the first appearance of yellows symptoms. Eleven trees in the positive index group showed ring spot as the first symptom after 1950, whereas 75 in the negative group showed ring spot as the first symptom after that year.

Table 2.—Sequence of ring spot (RS) and yellows (Y) symptoms in the Horn Farm Montmorency cherry orchard.

Year of first-appearance of any symptom	Ring spot first[a]			Yellows first[a]	
	No. trees	No. with Y in later years	R and Y in same year[a]	No. trees	No. with RS in later years
1945	0		0	5 (0)	0
1949	3	0	0	0	
1950	15 (2)	6 (1)	1 (0)	5 (0)	1 (0)
1951	36 (33)	19 (17)	6 (3)	8 (1)	3 (1)
1952	29 (28)	10 (10)	9 (6)	0	
1953	6 (5)		0	0	

[a] No. of trees of indicated category that indexed negative in 1950 are in parentheses beside the total for the category.

If, as seems likely, all except 5 of these 139 trees were virus-free at planting, the apparent incidence of yellows increased from 3.6% to 49.6% in 4 years, 1950 to 1953 (Table 3). The total diseased trees increased from 3.6% to 97.8% in 5 years, 1949 to 1953. All but 3 trees had expressed symptoms or indexed positive in 1950 or in 1952, at which time all trees not yet known to be diseased were re-indexed. Although the orchard contained at least a few infected trees as early as 1945, neither virus spread appreciably until 1949 or 1950. Then spread was very rapid.

There appeared to be a grouping of the trees with yellows, but rather even distribution of the trees with ring spot (Fig. 1).

Influence of yellows and ring spot on growth, fruit quality, and yield.—The trees were grouped by the year that yellows and ring spot symptoms first appeared on each tree. For each year from 1950 through 1952, the average yield of each group was compared with that of healthy trees in the orchard (Table 4).

Table 3.—Cumulative numbers of trees in the Horn Farm Montmorency cherry orchard that showed ring spot and yellows symptoms.[a]

Year	Ring spot % [b]	Yellows % [b]	Trees that indexed positive, with no symptoms to year indicated[c] % [b]	Total known diseased to year indicated[d] % [b]
1945		3.6		3.6
1949	2.2	3.6		5.8
1950	13.7	7.9	17.3	38.1
1951	44.6	19.4	7.9	64.7
1952	73.4	35.3	10.8	95.0
1953	78.4	49.6	9.4	97.8

[a] No additional symptomatic trees were found in surveys made in 1946 and 1947. No survey was made in 1948.

[b] Calculated on basis of 139 trees in the study.

[c] Indexing in 1950 and 1952.

[d] The total number of diseased trees is not always equal to the sum of the totals for ring spot, for yellows, and for trees that indexed positive because in many cases the same tree showed both symptoms in the same or in different years.

Crop data are not included for 1953, when poor pollination weather caused poor fruit set.

In 1950, trees showing ring spot for the first time yielded substantially less than healthy trees; in 1951 the difference in yield was even greater. In 1952, however, diseased trees bore as much fruit as healthy trees.

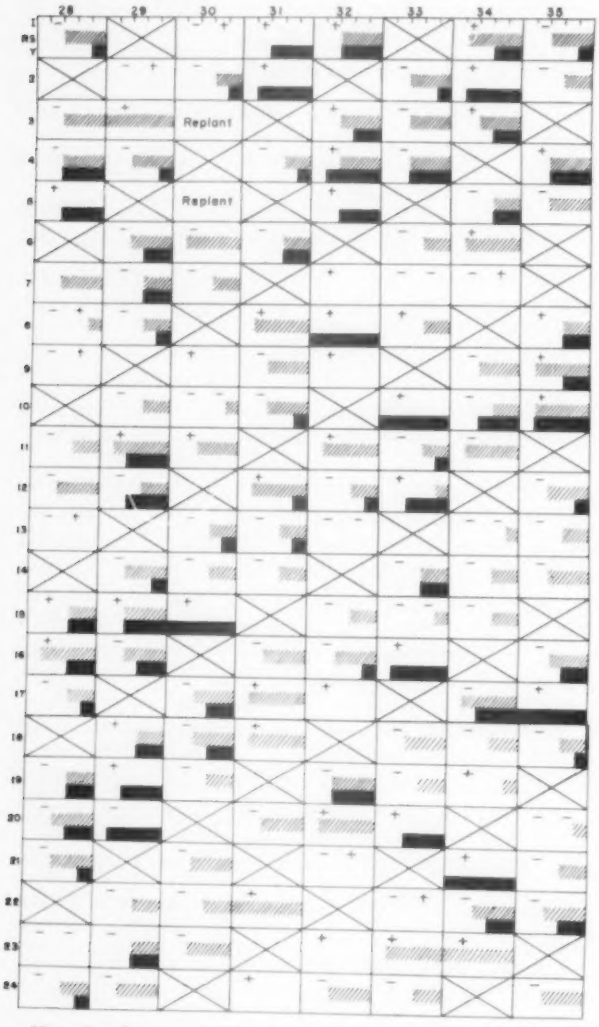

Fig. 1.—Diagrammatic chart of trees in the "C" block of the sour cherry orchard on the Horn Farm, Sodus, N. Y. Each rectangle represents a single tree. A plus (+) or minus (−) sign toward the left indicates a positive or negative test in 1950. Similarly, indexing results in 1952 are indicated toward the right at the top of the rectangle. No sign on the right indicates that no test was made in 1952. Note that the rectangle in the upper left hand corner is marked. I indicates the line for indexing, RS the line for ring spot, and Y the line for yellows.

Ring spot's first appearance is indicated by diagonal-line shading, as follows: entire line shaded, 1949; four-fifths, 1950; three-fifths, 1951; two-fifths, 1952; and one-fifth, 1953. Yellows symptom expression is shown by solid shading. The year representation is the same as for ring spot except that the entire line shaded indicates that symptoms were first observed in 1945.

The chart shows that yellows symptoms were observed on 5 trees in 1945. No additional symptomatic trees (either yellows or ring spot) were observed in 1946 or 1947, and no survey was made in 1948.

In 1951 trees showing ring spot for the first time yielded less than did healthy trees, but yields in 1952 were the same for both groups. In 1952, the appearance of first-year shock symptoms of ring spot was again associated with yields below normal; such trees yielded less than did the remaining healthy trees and also less than did the trees that had shown initial shock ring spot in either of the 2 preceding years. In any given year, trees that eventually developed shock ring spot symptoms but had not yet done so produced crops equal to those of the trees that remained healthy throughout the study.

On many of the trees classified as having yellows, shock ring spot symptoms preceded or accompanied the first expression of yellows, undoubtedly complicating yield data. Nevertheless, it is clear that yields remained lower on those trees than on the healthy trees.

The ring spot trees included in the yield study had smaller fruit than healthy trees in the year that symptoms first appeared (Table 5), but not in subsequent years. The yellows-affected trees had larger fruit than did the healthy trees in 1950 and 1951.

Fruit soluble solids and trunk circumference were measured in 1950, 1951, and 1952, but appeared unaffected by either disease.

Evidence was obtained in 1952 that terminal size was affected (Table 6). Only trees that first developed symptoms—either ring spot or yellows—that year had terminals smaller than those of healthy trees. Trees that developed symptoms before 1952 had larger terminals than did healthy trees.

Data on blossom set were also obtained in 1952

Table 4.—Yield of Montmorency sour cherry trees classified according to the year in which first symptoms of yellows (Y) or of ring spot (RS) were observed.

Symptoms and year of first appearance	No. of trees	Average yield/tree (lb.)[a]			Percentage of previous year[b]	
		1950	1951	1952	1951	1952
None	11[c]	131.2	133.3	144.1	101.6	108.1
RS '52	23[d]	*135.6*	*140.5*	114.7	103.6	81.6
RS '51	34[e]	*134.9*	101.9	156.9	75.5	154.0
RS '50	12[f]	96.4	76.5	149.4	79.4	195.3
Y '52	20[g]	*115.5*	*105.9*	140.0	91.7	132.2
Y '51	11	*111.8*	97.6	132.0	87.3	135.2
Y '50	6	64.7	72.9	117.7	112.7	161.5
Y '45	5	65.8	81.8	109.8	124.3	134.2

[a] Figures in italics are yields for years before symptoms listed appeared in the indicated trees. In years previous to the year indicated for first appearance of symptoms, yields on yellows trees were in many cases also affected by ring spot.

[b] Calculated from the average figures as listed in the table.

[c] Four trees indexed positive in 1952, but average yield in 1952 is the same with or without these 4 trees.

[d] Eight trees showed yellows in 1953. One tree indexed positive in 1950.

[e] Eight of these trees showed yellows symptoms also in 1952, and 9 in 1953. Two of the former and one with no yellows symptoms indexed positive in 1950.

[f] One tree showed yellows in 1951, and 3 in 1952.

[g] All these trees had expressed ring spot previously or in the same year.

Table 5.—Comparative size of fruit on ring spot (RS), yellows (Y), and healthy Montmorency cherry trees.

Symptoms and year of first appearance	No. of trees	Weight of fruit (g)[a]			Percentage of previous year[b]	
		1950	1951	1952	1951	1952
None	11	446.0	470.0	444.3	105.4	94.5
RS '52	23	*421.8*	*475.6*	439.1	112.8	92.3
RS '51	34	*461.3*	422.9	435.1	91.7	102.9
RS '50	12	427.0	504.0	437.0	118.0	86.7
Y '52	20	*422.8*	*474.6*	436.7	112.3	92.0
Y '51	11	*424.5*	501.6	447.4	118.2	89.2
Y '50	6	469.8	513.8	473.0	109.4	92.1
Y '45	5	491.3	529.4	437.2	107.8	82.6

[a] Average of weights of 100-fruit samples harvested at random from each tree. Italics indicate data for years previous to first appearance of the symptoms indicated.

[b] Calculated from average figures as listed in the table.

(Table 6). Percentage of set was greater on healthy trees than on any of the other groups, and percentage of set was lower on trees with yellows than on trees with ring spot. Greater differences in the same direction have been reported from New York (1).

Discussion.—The indicated rapid spread of ring spot virus is probably related to an easy dissemination by an arthropod vector. The lack of definte pattern indicates a possible strong-flying vector or one that moves readily over appreciable distances. The grouping of yellows-affected trees in the orchard suggests that a local source of inoculum might have been present. These data are in agreement with the more extensive data of Willison et al. (9). This study agrees with the suggestion of Willison et al. (9), that the ring spot virus has a "wider range of spread than yellows."

The effect of yellows on yield reported here is not as great as that reported by Moore (7). It is, however, in substantial agreement with the report of Keitt and Clayton (2) with respect to both the net effect on yield and the progressively greater effect following the appearance of symptoms. These results are also in general agreement with those of Lewis (5) with respect to the effects of both yellows and ring spot on yield.

The increased fruit size on yellows trees over fruit on healthy trees in years following initial infection is probably due to less growth of fruit spurs and to less fruit set on diseased trees than on healthy trees. The larger terminals on yellows-affected trees than on healthy ones in subsequent years may be explained by the fewer shoots per tree.

Percentage of fruit set was lower on yellows-affected trees and ring-spot-affected trees than on healthy trees. Yellows-affected trees lose a large number of leaves early in the season, which undoubtedly influences fruit bud formation for the next season and probably accounts for the poor fruit set. Certainly it would be expected that the loss of leaves early in the season would reduce the amount of accumulated carbohydrates, which are necessary to good fruit set in the following season. — Department of Plant Pathology, Cornell University, Ithaca, New York.

Table 6.—Terminal growth and fruit set of Montmorency cherry trees classified according to the year in which symptoms of ring spot (RS) and of yellows (Y) first appeared.

Symptoms and year of their first appearance	Measurements of terminals in 1952[a]				
	No. of trees	Length (cm)	Diameter[b] (cm)	Volume[c] (cm^3)	Percentage[d] set (1952)
None	11	13.4	0.225	0.625	23.4
RS '52	23	12.2	0.221	0.584	17.5
RS '51	32	14.8	0.227	0.738	18.5
RS '50	12	13.9	0.228	0.669	18.2
Y '52	20	11.1	0.209	0.545	17.3
Y '51	11	14.8	0.244	0.811	16.7
Y '50	6	15.9	0.245	0.824	15.3
Y '45	5	17.9	0.252	1.028	15.5

[a] Three shoots selected at random within each of 4 sectors of the tree and an average of the 12 shoots determined for each tree.

[b] Measured midway from base to tip of the shoot.

[c] Calculated from length and diameter measurements on the assumption that the diameter measurement was the mean for the entire length of the terminal. Figure listed is an average volume determined from 12 twigs in each tree, calculated, from the length and diameter, by the formula $\pi r^2 \times$ length.

[d] Blossoms on 2 twigs each 1 cm in diameter, counted on each tree at blossom time, and fruit set counted about 1 month later. Figure is average of percentage set determined for each tree.

LITERATURE CITED

1. Cain, J. C., and K. G. Parker. 1951. A preliminary report on the response of virus-infected Montmorency cherry trees to nitrogen fertilizer. Phytopathology 41:661-664.

2. Keitt, G. W., and C. N. Clayton. 1943. A destructive virus disease of sour cherry. Phytopathology 33:449-468.

3. Keitt, G. W., and J. D. Moore. 1943. Masking leaf symptoms of sour cherry yellows by temperature effects. Phytopathology 33:1213-1215.

4. Lewis, F. H. 1947. Notes on two virus diseases of sour cherries. Proc. State Hort. Assoc. Penn. 24:65-71.

5. Lewis, F. H. 1951. The effect of ring spot and yellows on the yield of Montmorency. (Abstr.) Phytopathology 41:24.

6. Mills, W. D. 1946. Temperature effects on the expression of the yellows virus in sour cherries. Phytopathology 36:353-358.

7. Moore, J. D. 1946. Relation of sour cherry yellows to yield. (Abstr.) Phytopathology 36:406.

8. Moore, J. D., and G. W. Keitt. 1946. Relation of temperature to expression of symptoms of sour cherry yellows and necrotic ring spot. (Abstr.) Phytopathology 36:406-407.

9. Willison, R. S., G. H. Berkeley, and G. C. Chamberlain. 1948. Yellows and necrotic ring spot of sour cherries in Ontario—distribution and spread. Phytopathology 38: 776-792.

HOST-PARASITE RELATIONS OF LEPTODISCUS TERRESTRIS ON ALFALFA, RED CLOVER, AND BIRDSFOOT TREFOIL

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SUMMARY

Small, brown, sunken, necrotic spots developed on leaflets of alfalfa, red clover, sweet clover, and birdsfoot trefoil inoculated with *Leptodiscus terrestris*. Small, black, elongate lesions were produced on petioles of alfalfa and sweet clover, and small reddish-brown lesions formed on birdsfoot trefoil. In these three species infected petioles abscissed from the stems. Infected red clover petioles were girdled and collapsed. The fungus caused blackening on the stems of the four legumes, and young stems of alfalfa and birdsfoot trefoil were killed. The fungus grew into alfalfa crowns through wounds, through young stems killed by the fungus, and through the vessels and tracheids of inoculated mature stems cut off above the infection. In uncut mature alfalfa stems, hyphae were restricted to the cortex by a periderm. In red clover stems no periderm formed, and hyphae were present in all tissue. The fungus entered red clover crowns through dead stems and wounds. In unwounded taproots of alfalfa and red clover, periderm formation in the secondary phloem prevented the fungus from reaching the xylem. The phloem of alfalfa taproots was penetrated only through wounds; the phloem of red clover was also penetrated through dead lateral roots. Periderm in the xylem was ineffective in restricting advance of the fungus in both alfalfa and red clover. Periderm formation in the secondary phloem of taproots was ineffective in preventing the advance of the fungus in birdsfoot trefoil.

Leptodiscus terrestris Gerd. (1) has been found associated with crown and root rot of forage legumes in central and southern Illinois since 1950. It is highly pathogenic on red clover, *Trifolium pratense* L., and other legumes (2). Though it caused damping-off and a root rot of alfalfa seedlings, *Medicago sativa* L. (2), its relationship to crown and root rot of mature alfalfa plants has not been studied. Spores are capable of infecting leaves and stems (2), but that stage of the life cycle was not found in the field.

This study was undertaken to determine: 1) how the fungus gains entrance into the crown and taproot of mature alfalfa, birdsfoot trefoil, *Lotus corniculatus* L., and red clover; 2) the part spores play in its life cycle; and 3) the relationship of leaf and stem infection to root and crown rot.

Materials and methods.—The soil used in all greenhouse experiments was a 3:1 mixture of soil and sand steamed 3 hours on each of 2 successive days.

Before inoculation with spore suspensions, leaves and stems were sprayed with a 0.5% Tween 20 solution and rinsed with distilled water. The plants were then sprayed with the spore suspension and placed in a moist chamber for 30 hours.

Mycelial-sclerotial suspensions used in inoculations were prepared by removing sclerotia and mycelium from potato-dextrose agar slants, with as little agar as possible, and mincing them in a Waring Blendor with sterile distilled water.

Occurrence of the pathogen.—In 1955, alfalfa, red clover, birdsfoot trefoil, and sweet clover, *Melilotus alba* Desr., were interseeded with corn on the Agronomy South Farm at the University of Illinois Agricultural Experiment Station. During the summer, 90–100% of the legume seedlings were damped-off by *Leptodiscus terrestris*. The fungus produced spores in abundance on the hypocotyls of dead seedlings. This is the first observation of sporulation of this fungus in nature.

In September, 1955, *L. terrestris* was found associated with a severe root and crown rot of alfalfa in southwestern Illinois. High percentages of plants in 2- to 3-year-old stands were infected, and the disease appeared to limit the profitable life of a stand to 4–5 years. External symptoms on the taproots were rough dark-brown lesions with black margins. Internal symptoms, in median longitudinal sections, were V-shaped dark-brown decay of the xylem, with black advancing margins (Fig. 1-A). Sclerotia were usually present in the brown decayed tissue. Alfalfa plants killed by Leptodiscus crown rot were found in stands 3 years old or more. Individual stems or entire plants wilted and died, and the leaves became light tan. In contrast to bacterial wilt symptoms, the top growth of infected plants did not appear stunted.

L. terrestris was isolated at monthly intervals for one year from the crowns, taproots, and base of dead stems of 1–5-year-old alfalfa plants collected at the Agronomy South Farm. In many instances, only *L. terrestris* grew from the black advancing margin of rot leading into the crown from dead stems of 1- and 2-year-old plants (Fig. 1-B). The fungus was also isolated from living blackened alfalfa stems. During the winter, dead alfalfa stems containing sclerotia were surface-disinfested with a 5% Clorox (sodium hypochlorite) solution, rinsed in distilled water, and placed on sterile moist filter paper in Petri plates. Sporulation occurred on these stems in 4–5 days.

In November, birdsfoot trefoil plants previously killed by crown rot were dug and placed so that the stems were in contact with the field soil surface. During the winter the fungus was isolated from sclerotia removed from the roots of these plants at monthly intervals.

Leaf and stem infection.—Leaves and stems of alfalfa, sweet clover, red clover, and birdsfoot trefoil were inoculated with spore suspensions. The first symptom of infection on alfalfa leaflets was a greenish-yellow pinpoint fleck. This fleck enlarged, became

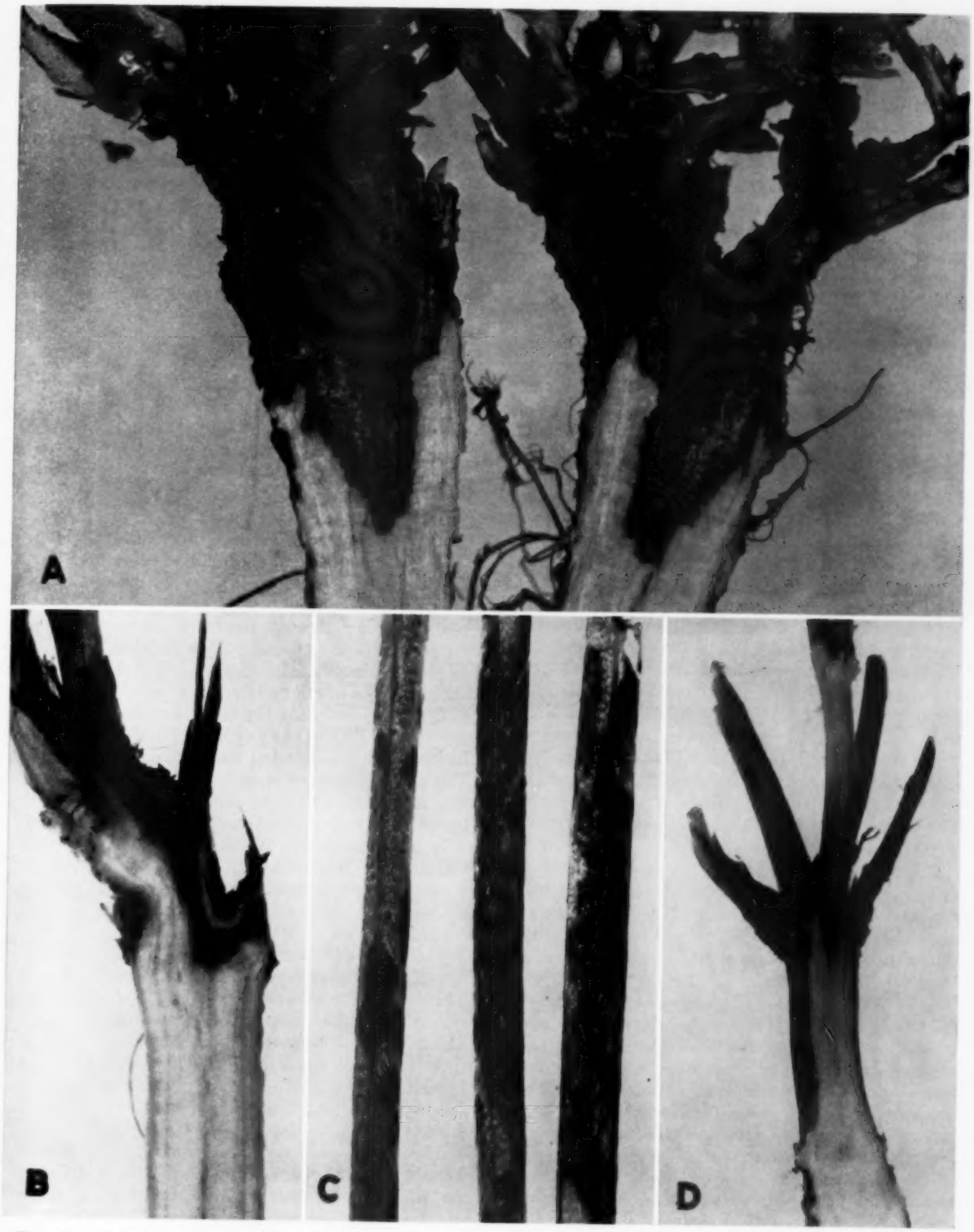

Fig. 1.—**A**) Field-collected alfalfa plant with an advanced stage of crown rot caused by *Leptodiscus terrestris*. **B**) Field-collected one-year-old alfalfa plant with crown rot caused by *L. terrestris*. The fungus appears to have invaded the crown through a dead stem. **C**) Ranger alfalfa stems artificially inoculated with a spore suspension of *L. terrestris*, showing blackening. **D**) Ranger alfalfa plant with an early stage of crown rot following stem inoculation with a spore suspension of *L. terrestris*. The stem was cut above the point of inoculation following infection.

brown and sunken, and was surrounded by a greenish-yellow halo. The individual spots, halo included, were about 1 mm in diameter. Symptoms on Israel and Evergreen sweet clover were similar to those on alfalfa except that the spots became slightly larger. On red clover and birdsfoot trefoil, the spots were reddish-brown, with the halo indistinct or absent. No sporulation occurred on infected leaves unless they were removed from the plant and incubated in a moist chamber. The fungus then grew from the necrotic spots throughout the leaf and began to form fruiting structures in about 3 days (3).

Twelve hours after inoculation, the spores had germinated and produced germ tubes and appressoria. Germ tubes usually grew from only one cell of the spore, but occasionally formed from both cells. They were usually short, and appressoria sometimes formed directly from the spores. The germ tubes occasionally branched and produced several appressoria, but they did not form on extremely long germ tubes. Penetration was predominantly direct but was also between epidermal cells or through stomata. Infection pegs penetrated either the cuticle or the cuticle and cell wall. After penetration, a vesicle formed from the infection peg, either between the cuticle and the cell wall or within the epidermal cell. One, two, or sometimes three hyphae grew from the vesicle. When the vesicle was between the cuticle and the cell wall, the hyphae penetrated the cell wall almost at once or forced their way across 2–4 cells before penetrating. When the vesicle occurred over the junction of 2 cells, one hypha occasionally penetrated intercellularly and another proceeded for some distance between the cuticle and cell wall before entering an epidermal cell. From the epidermal cell, the hyphae grew intercellularly into the interior of the leaflet, branching sparsely and continuing through the leaflet to the opposite side. The hyphae ceased growth in 5–6 days and remained restricted to the necrotic tissue.

The fungus produced small, black, elongate lesions on the petioles of alfalfa and sweet clover, and similar reddish-brown lesions on birdsfoot trefoil, causing the petioles to abscis. Severely infected red clover petioles were girdled, and the leaves died without an abscission layer forming.

L. terrestris caused a blackening of the stems of alfalfa (Fig. 1-C), sweet clover, red clover, and birdsfoot trefoil. Symptoms on alfalfa ranged from the killing of young stems to small, elongate, black lesions on mature stems. When several points of infection were close together they coalesced and the blackened area became sunken and extended longitudinally, often encircling the stem. Symptoms on birdsfoot trefoil and sweet clover were similar to those on alfalfa except that the lesions were first reddish-brown, later becoming black. Young stems were killed in birdsfoot trefoil but not in sweet clover.

Stem infection was more extensive on red clover than on the other legumes, and stems were consequently examined histologically. The hyphae were intracellular and present in all tissues. No periderm formed in the cortex. The epidermal, collenchyma, and underlying thin-walled parenchyma cells were filled with mycelium. Hyphae formed a swelling in contact with the cell walls, and a hyphal peg penetrated the wall. Once through the wall, the hyphae enlarged. In vessels and tracheids, hyphae formed swellings against the cell plate or wall, and hyphal pegs passed through the pits and formed swellings on the other side of the walls. Greatest longitudinal growth was through the pith.

Root and crown infection.—Greenhouse-grown Ranger alfalfa plants were inoculated with *Leptodiscus terrestris* to determine if it would grow from infected stems into the crown. One stem on each of 20 plants was wrapped with aluminum foil, and the lower portion was sealed with petroleum jelly. A spore suspension was injected between the stem and the aluminum foil with a hypodermic syringe, and the plants were placed in a moist chamber for 2 days. Two weeks later the aluminum foil was removed. One month after inoculation, 10 of the stems were cut above the infected area. Uninoculated controls were maintained for both treatments.

Four months after inoculation, the infected areas on the stems were black. Three of the 10 uncut inoculated stems were killed, and the decay had advanced into the crown. The interior of the remaining 7 uncut inoculated stems was healthy. Infection on 4, however, had progressed through the cortex of the stems onto the surface of the crown; on 3 it had not reached the crown. On inoculated cut stems, the fungus had progressed to the interior of the stems and into the crown of 6 plants, producing the characteristic V-shaped decay (Fig. 1-D). In the remaining 4 stems, the fungus had entered the stele but had not reached the crown. The uninoculated cut stems were tan, and the interior was reddish-brown at the junction of living and dead tissue. The uninoculated uncut stems were healthy.

In uncut stems not killed by the fungus, the mycelium grew intracellularly through the cortex until it reached a periderm. In cut stems, hyphae penetrated the vascular tissue and advanced through the xylem toward the crown.

The roots and crowns of 10-month-old greenhouse-grown alfalfa plants were inoculated with *L. terrestris*. The roots were washed free of soil and dipped in a sclerotial-mycelial suspension of the fungus and replanted in fresh steam-sterilized soil in 8-in. pots at the rate of 4 plants per pot. Forty plants were inoculated; 20 uninoculated plants were controls. The top growth was removed periodically, and the experiment was ended after 14 months.

The crowns and taproots of control plants remained healthy, and the junction between living and dead tissue in cut stems was a light brown. A dark-brown to black rot was present on the crown and roots of all inoculated plants. Many secondary and tertiary roots turned black and died, and contained sclerotia. In severe cases the taproots were girdled and roughened with fissures. In less severe cases lesions on the taproots were confined to areas where laterals emerged.

In no taproot, however, did decay extend through the periderm and secondary phloem into the xylem (Fig. 2-A).

The lower portion of some stems was black, and a few buds were killed. The fungus grew into the cut stems that had partially died-back. The interior of such stems was brown to black, with the most advanced point of infection in the pith. Sclerotia were often present in the dead tissues. Decay from some of these stems extended into the xylem of the crown and taproot.

In another experiment, *L. terrestris* was tested for its ability to cause rot in wounded and unwounded alfalfa crowns. One month before inoculation, one-year-old greenhouse-grown alfalfa plants were transplanted singly to 6-in. pots. The crown and large woody stems were stabbed with a sterilized needle, and sclerotial-mycelial suspension was poured over wounded and unwounded plants. There were 6 plants per treatment, and uninoculated controls were maintained for both treatments.

Five months later, rot was present on the surface of the crown and upper portions of the taproot of all inoculated plants. The lower portions of many living

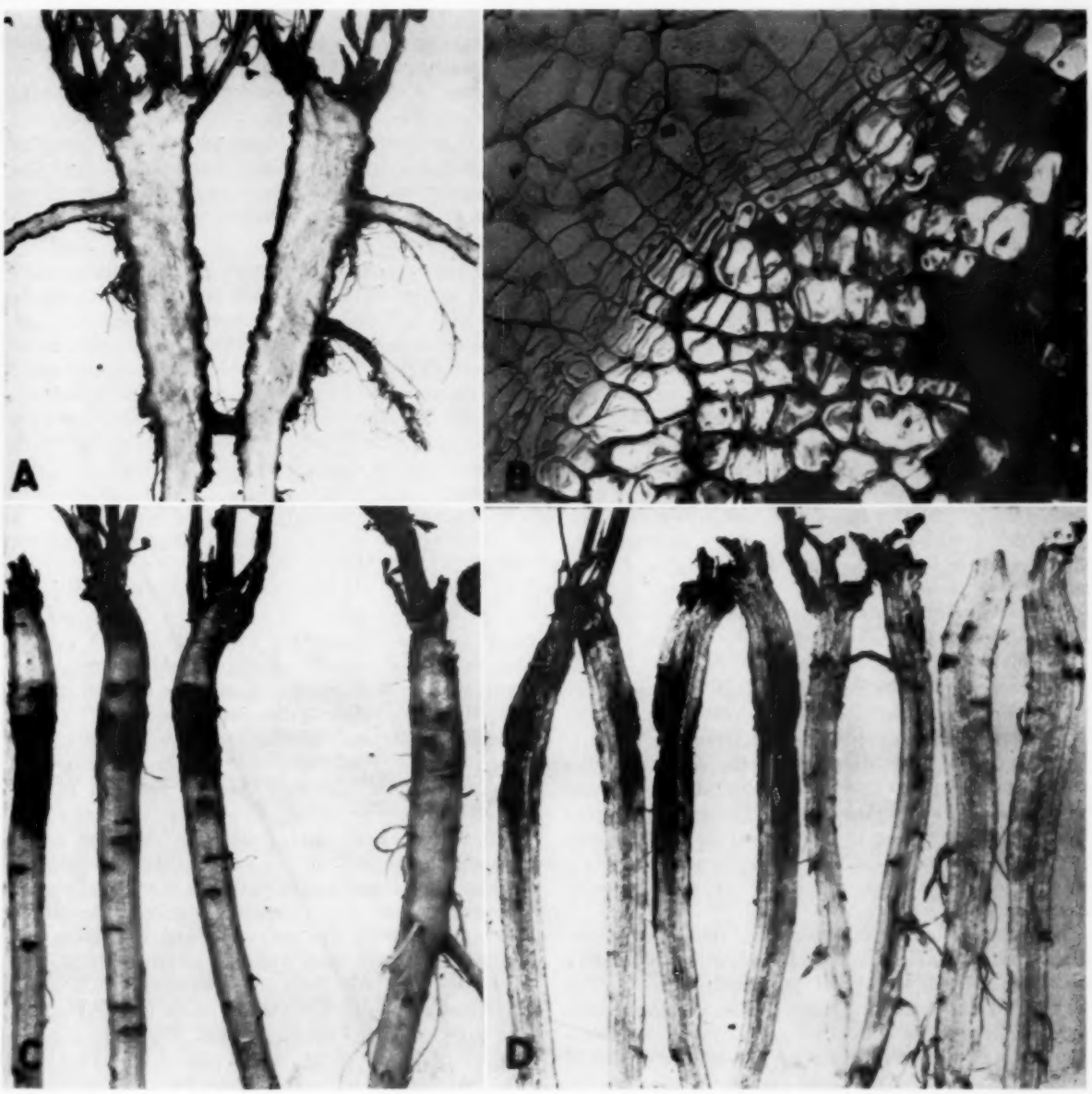

Fig. 2.—Ranger alfalfa roots artificially inoculated with *L. terrestris*. **A)** Longitudinal section of an unwounded taproot 14 months after inoculation. The decay is restricted to the periderm and secondary phloem. **B)** Periderm separating infected and healthy cells in an inoculated unwounded root (×270). **C)** External appearance of taproots 39 days after wound inoculation. Inoculated, 3 roots at left; uninoculated control, root at right. **D)** Internal appearance of taproots 39 days after wound inoculation. Inoculated, 4 sections at left; uninoculated control, 4 sections at right.

stems were black, and sclerotia were present in stems that had died as a result of cutting. In unwounded plants, decay in the xylem was traceable only to dead stems. Rotted areas in the xylem of the crowns of wounded plants were traceable to dead stems or wounds. Rot extending into the crown from dead stems had the characteristic V-shaped black advancing margin, whereas rotted tissue traceable to wounds had a more irregular shape.

The crown and taproot of both wounded and unwounded control plants appeared healthy. The tissue around the wounds was reddish-brown, and there were some reddish-brown streaks in the xylem. In both types of controls, the margin between dead and living stem tissue was usually tan; occasionally it was black. Isolation, however, yielded only *Fusarium solani* (Mart.) App. & Wr., *F. oxysporum* Schlecht., *F. moniliforme* Sheldon, *Alternaria* sp., and *Myrothecium* sp.

Wounded and unwounded taproots of alfalfa, red clover, and birdsfoot trefoil were inoculated with *L. terrestris*. Roots of 5-month-old alfalfa, 6-month-old red clover, and birdsfoot trefoil were washed free of soil, and a portion of the taproot just below the crown was washed with a 5% Clorox solution, rinsed with distilled water, and wounded by stabbing with a flamed needle. Sterile gauze was dipped in a sclerotial-mycelial suspension and wrapped around wounded and unwounded roots. Roots of wounded and unwounded control plants were wrapped with sterile gauze. The plants were replanted in steam-sterilized soil, 4 plants per pot, and the experiment was replicated 6 times. The roots were examined 39 days after inoculation.

The decayed areas around the wounds on alfalfa roots were irregular in shape and dark brown with narrow black margins. When severe decay encompassed the taproot, the lesion was sunken and rough and many lateral roots were killed (Fig. 2-C). Decay entered the xylem through wounds that penetrated the phloem and progressed vertically through the vascular tissue (Fig. 2-D). The advancing margin of decay was black, and the decayed tissue was brown and contained sclerotia. There were also some reddish-brown streaks in the xylem in advance of the black margin of decay. The exterior of unwounded inoculated roots had the same general appearance as the wounded inoculated roots. The point of attack was the spongy parenchyma cells around the base of the lateral roots. The rot, however, was confined to the periderm and secondary phloem, and there was no decay or reddish-brown streaking in the xylem.

The exterior lesions on wound-inoculated red clover roots were dark brown with a black margin. Secondary roots were killed, and many contained sclerotia. The rot girdled the taproots of most plants, and the lesions were rough and cracked, with some of the rotted tissue sloughing away. Sunken areas occurred at points of wounding, and sclerotia were present in the decayed tissue of severely affected roots. The decay extended through the wounds into the xylem, where it made its greatest longitudinal progress. Considerable reddish-brown streaking was also present throughout the xylem of the taproot and crown. One plant was killed, and the lower portion of one taproot rotted off. In 2 plants the rot also entered the crown through a dead stem. Unwounded inoculated roots had the same external appearance as wounded roots. Decay, however, was confined to the periderm and secondary phloem except in a few cases, where it had entered the xylem through dead lateral roots.

The exterior of both wounded and unwounded birdsfoot trefoil roots was dark reddish-brown, and sunken areas occurred where they had had been wounded. Many lateral roots were killed, and on many plants the rot extended upward over the crown and base of the stems. The fungus entered the taproots through wounds, and its most rapid advance through the xylem was longitudinal. The fungus also grew through the secondary phloem of unwounded taproots and invaded the xylem. The lower portion of the taproot was rotted off of 9 wounded and 4 unwounded plants, and new roots had developed from the crowns. Birdsfoot trefoil appears to be much more susceptible than either alfalfa or red clover to root and crown rot caused by *L. terrestris*.

The area around the wounds of uninoculated roots of all 3 species was slightly sunken and a light reddish-brown. This discoloration extended through the secondary phloem, and reddish-brown streaks were present in the xylem. The unwounded uninoculated roots were healthy and free of discoloration in the xylem.

Paraffin sections were made of wounded and unwounded inoculated roots, wounded uninoculated roots, and portions of inoculated alfalfa crowns bearing dead stems.

In wounded uninoculated roots of all 3 species, a periderm formed in the secondary phloem around the wound.

In the crown and taproot of inoculated alfalfa the hyphae were intracellular, with a sharp boundary between affected and healthy cells. Hyphae in contact with cell walls formed a swelling, and a hyphal peg penetrated the wall and enlarged again on the opposite side. Infected xylem cells became plugged with a granular substance. In alfalfa the fungus grew from dead stems into the crown. At the transition region of stem and crown, phellogen formed between the infected and healthy tissue. It was not effective in retarding the fungus, and hyphae entered the crown through vessels and tracheids. Behind the advancing margin, the mycelium spread into the thick-walled parenchyma and ray parenchyma cells. The parenchyma cells were killed and contained a dark-staining granular material. Some vessels and parenchyma cells were filled with mycelium and sclerotia.

Hyphae also entered the xylem of the alfalfa taproots through wounds and progressed in a longitudinal direction. In the wounded secondary phloem, the dead cells were separated from healthy cells by a periderm that was not invaded by the fungus. Regions were found in the xylem also where a periderm separated infected tissue from healthy tissue; restriction of the growth of the fungus was not as effective in the xylem as in the

phloem, however.

On unwounded inoculated alfalfa roots, lesions starting in the secondary phloem were confined to that tissue, and there was a well-defined periderm between affected and normal cells (Fig. 2-B).

Host-parasite relations were similar in red clover and birdsfoot trefoil to those in alfalfa. In red clover a periderm usually formed in the secondary phloem between infected and healthy cells; restriction of the growth of the fungus was not as effective as in alfalfa, however. The hyphae continued to advance, and another periderm usually formed, deeper in the phloem. In birdsfoot trefoil also, periderm formed in the secondary phloem, but it did not form a continuous zone between invaded and healthy cells. It was ineffective in walling off the fungus, and hyphae grew through the phloem into the xylem.

Discussion.—Although *Leptodiscus terrestris* is capable of infecting leaves, stems, crowns, and roots, only crown and root rot appear to cause serious damage to the hosts. Leaf spot may be important in that infected leaves fall to the soil surface where spores and sclerotia may form. This leaf spot has not been recognized in the field since it would be very difficult to distinguish morphologically from the early stages of other leaf diseases. The black stem disease caused by *L. terrestris* can only be distinguished from other black stem diseases by isolation. Stem infection is important in that the fungus may grow into the xylem of the stem and hence into the xylem of the crown when the stems are cut for hay or the field is pastured.—Department of Plant Pathology, University of Illinois, Urbana.

LITERATURE CITED

1. Gerdemann, J. W. 1953. An undescribed fungus causing a root rot of red clover and other Leguminosae. Mycologia 45:548-554.

2. Gerdemann, J. W. 1954. Pathogenicity of Leptodiscus terrestris on red clover and other Leguminosae. Phytopathology 44:451-455.

3. McVey, D. V., and J. W. Gerdemann. 1959. The morphology of Leptodiscus terrestris, and the function of setae in spore dispersal. Mycologia (in press).

REVERSAL OF FUNGITOXICITY OF 8-QUINOLINOL BY AMINO ACIDS AND OTHER CHELATORS

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SUMMARY

Amino acids and other chelators were tested for their ability to reverse the toxicity of 8-quinolinol (oxine) to spores and mycelium of *Aspergillus niger* and to mycelium of *Botryosphaeria ribis*. In the spore test with *A. niger*, cysteine, histidine, tryptophane, casamino acids, dithizone, and versene reversed oxine toxicity, and glutamic acid, asparagine, and glutathione did not. In the mycelium test with *A. niger*, cysteine reversed oxine toxicity; glutathione, asparagine, histidine, and tryptophane had no effect; and glutamic acid made the oxine more toxic. In the *B. ribis* test, cysteine reversed oxine toxicity, and glutathione, asparagine, histidine, tryptophane, glutamic acid, glycine, and methionine had no effect. Dithizone and quinaldic acid reversed the toxicity of oxine to the spores of *Stemphylium sarcinaeforme* and *Monilinia fructicola*. *In vitro* studies showed that 0.5% solutions of histidine and cysteine can remove copper from a 7-ppm solution of copper oxinate. It is suggested that copper oxinate produces fungitoxicity in the following manner. The amino acids of the cell take copper from the half-chelated copper oxinate, and release oxine *in situ*. The copper "poisons" amino acids, proteins, and enzymes while the freed oxine sequesters prosthetic trace metals such as Fe^{++}, Zn^{++}, and Co^{++}. How these reactions can occur is explained on the basis of the relative stability constants of the various systems involved, and the relative concentrations within the cell of the component parts of these systems.

In 1943 Zentmyer proposed that 8-quinolinol (oxine) is fungitoxic because it is a powerful chelating agent and may act by robbing fungi of required metal nutrients (10, 11). That the fully chelated copper oxinate $Cu(Ox)_2$ is more toxic than oxine itself (4, 8) apparently contradicted the "metal robbing" theory. This implies that oxine added to the ordinary medium containing trace amounts of metals must form $Cu(Ox)_2$ to be toxic at very low dosages. Further data on this point (3, 4) show that fungitoxicity is reduced by any conditions interfering with the ability of oxine to form $Cu(Ox)_2$ or reducing the amount of $Cu(Ox)_2$ in the system.

Surprisingly, an excess of oxine also reduces the fungitoxicity of $Cu(Ox)_2$ (4). An explanation is offered by the hypothesis of Albert et al (2), who suggested that the actual toxic form of metal (Me^{++}) oxinate is the half chelate, $Me^{+}Ox$, which is formed by dissociation of $Me(Ox)_2$ within the organism. The excess oxine could lower toxicity by entering the organism and, following the law of mass action, reducing the amount of $Me^{+}Ox$ formed by dissociation at the site of toxic action. The amount of $Me^{+}Ox$ outside the organism could not be directly related to toxicity, apparently because its poor lipid solubility slows or prevents its permeation into the organism.

The factors considered in studying the toxicity of oxine to fungi and bacteria are the stability constants of its metal chelates, their degree of ionization, and their relative solubilities in lipids or nonpolar organic

Table 1.—Effect of cysteine, histidine, and casamino acids on the toxicity of oxine to spores of Aspergillus niger.

Material added	Spore germination at various concentrations (ppm) of oxine[a]						
	0	1.25	2.5	5.0	10	20	40
None	+	0	0	0	0	0	0
L-cysteine (100 ppm)	+	0	0	0	0	0	0
L-cysteine (1000 ppm)	+	+	+	0	0	0	0
L-cysteine (5000 ppm)	+	+	+	+	+	+	0
L-histidine (1000 ppm)	+	0	0	0	0	0	0
L-histidine (5000 ppm)	+	+	+	+	0	0	0
Casamino acids (2.0%)	+		+	+	0	0	

[a] + = spores germinated; 0 = spores not germinated.

solvents and in water. Block (4) showed that, though all these factors are important, no one of them can be strictly related to the fungitoxicity of $Cu(OX)_2$. Block (4) and Horsfall (6) suggested that copper removed from copper oxinate within the organism by naturally occurring chelating agents poisons vital cell constituents. The oxine thus freed is able to inhibit metal-requiring enzymes, presumably by robbing them of their metallic prosthetic groups.

Byrde and Woodcock (5) showed that a chelating agent, the disodium salt of ethylenediaminetetraacetic acid, reversed the fungitoxicity of oxine but increased the fungitoxicity of 5-n-amyloxine and 5-phenyloxine. Sijpesteijn et al (9) reported that the chelator sodium dibutyldithiocarbamate reversed the fungitoxicity of oxine.

A series of experiments was devised to see whether strong naturally occurring chelators such as cysteine and histidine (1) and other chelators can indeed take copper from oxine. If this should prove true, overloading the system with chelators other than oxine would reverse the toxicity of oxine, presumably by reducing the amount of chelate complex to less than a toxic level. This paper expands a preliminary note (12), presenting details and further information.

Materials and methods.—Tests were run primarily in a synthetic agar (Czapek's), with the potential antagonists added before autoclaving the medium. Stock solutions of oxine were prepared in flasks of autoclaved ion-exchange water, and the fungicide was pipetted with sterile pipettes into the flasks of melted agar just before plates were poured.

In most tests, plates were inoculated in 3 ways: 1) with a spore drop of *Aspergillus niger* Van Tiegh spores to test the effect of treatments on spore germination, 2) with a 5-mm disk from a potato-dextrose-agar culture of *A. niger* containing both mycelium and spores, and 3) with a 5-mm disk from a potato-dextrose culture of *Botryosphaeria ribis* Grossenb. & Duggar containing mycelium only. The effect of various treatments on spore germination was recorded merely by visual examination of the plates after incubation for 72 hours and for 7 days. Effect on the other inoculum was determined by measuring diameters of colonies developing from disks after the same incubation periods.

The pH of the autoclaved agar media was 6.5. Inoculum was taken from actively growing mycelium at the margin of Petri-dish cultures.

Amino acids used were: casamino acids, glutathione, L-cysteine, glutamic acid, L-asparagine, DL-tryptophane, L-histidine, glycine, and DL-methionine.

Results.—Preliminary tests showed that casamino acids, histidine, or cysteine strongly reversed the fungicidal action of oxine on the germination of *A. niger* spores (Table 1). Of the three, cysteine was by far the most effective for reversing the toxicity of oxine to the germination of *A. niger* spores on agar.

Other naturally occurring chelators were compared with cysteine in the *A. niger* spore test on agar. Glutamic acid was chosen because it is the principal amino acid components of the casamino acid hydrolysate used in the first experiment. Glutathione was chosen to see if the toxicity reversal by cysteine was related to the possession of sulfhydryl groups. Asparagine and tryptophane were included as two additional types. The results (Table 2) show that glutamic acid, glutathione, and asparagine do not reverse the fungitoxicity of oxine, and that tryptophane does so only slightly.

The materials shown in Table 2, plus histidine, were also tested for their ability to reverse the toxicity of oxine to the mycelial growth of *A. niger*. All the compounds were tested at 5000 ppm. None of the added compounds but cysteine were reversing. Glutamic acid apparently increased the toxicity of oxine to the mycelial growth of *A. niger*. *A. niger* mycelium grew at 20 ppm oxine without glutamic acid, but did not grow at 5 ppm oxine with added glutamic acid. Glutamic acid at 5000 ppm is not toxic to *A. niger* mycelium without oxine.

The materials in Table 2 were compared with glycine, histidine, and methionine for ability to reverse the toxicity of oxine to the mycelial growth of *Botryosphaeria ribis*. Glycine was chosen as the simplest amino acid, and methionine as another sulfur-containing amino acid. All were tested at 5000 ppm. Again, only cysteine reversed the toxicity of oxine.

Table 2.—Effect of naturally occurring chelators on the toxicity of oxine to spores of Aspergillus niger.

Material added at 5000 ppm	Spore germination at various concentrations (ppm) of oxine[a]					
	0	0.62	1.25	2.5	5	10
None	+	0	0	0	0	0
L-cysteine	+	+	+	+	+	+
Glutamic acid	+	0	0	0	0	0
Glutathione	+	0				
L-asparagine	+	0	0	0	0	0
DL-tryptophane	+	+	0	0	0	0

[a] + = spores germinated; 0 = spores not germinated.

The synergistic effect of glutamic acid on the toxicity of oxine to the mycelium of *A. niger* was not evident in the test using *B. ribis*.

To see whether the oxine toxicity reversal is related to the ability of a compound to chelate, the synthetic chelators dithizone (diphenylthiocarbazone) and versene (ethylenediaminetetraacetic acid) were tried in the *A. niger* spore germination test. The results (Table 3) show that both dithizone and versene are able to reverse the toxicity of oxine, with dithizone the more effective. In additional tests on glass slides, dithizone (10,000 ppm) and quinaldic acid (quinoline-2-carboxylic acid) (1000 ppm) reversed the toxicity of oxine to the germination of spores of *Stemphylium sarcinaeforme* (Cav.) Wilts, and *Monilinia fructicola* (Wint.) Honey, two fungi commonly used in testing fungicides.

To test whether the amino acids can actually take copper from copper oxinate *in vitro*, water solutions of histidine and cysteine, both at 5000 ppm, were shaken in separatory funnels with a chloroform solution of copper oxinate at 7 ppm. Equal volumes of amino acid solutions and copper oxinate solutions were used. The amount of copper oxinate remaining in the chloroform after it was shaken with the amino acids was determined by ultraviolet spectrophotometry, with absorption at 410 mμ. Shaking with histidine reduced 7 ppm copper oxinate to 2.2 ppm. Shaking with cysteine reduced it to 1.3 ppm.

Discussion.—To interpret the results reported here it must be assumed that the toxicity of very low concentrations of oxine is governed and limited by the small amount of copper present as a micronutrient. The effectiveness of cysteine and histidine as antagonists of oxine correlates well with the ability of these amino acids to bind metals. Albert (1) found that cysteine and histidine were the two strongest chelators of the amino acids he tested for their ability to bind divalent nickel or zinc. Our *in vitro* experiments demonstrated that either histidine or cysteine, when greatly in excess, can take copper from $Cu(Ox)_2$. Naturally occurring chelators can then influence the interaction of copper with oxine and thus modify the biological activity of copper oxinate.

The stability constants for $Cu(Ox)_2$ and the copper complex of histidine are respectively 29 and 18.3 (7). Therefore, outside the cell, tremendous quantities of cysteine or histidine should be required to remove copper from the 1:2 complex. That this was true is shown by the requirement in the spectrophotometric studies of 5000 ppm for reversal and for removing copper from copper oxinate.

Our data presumably result from interactions outside the organism, but they help to explain how copper oxinate may act within the cell. The theory accepted at present is that copper oxinate enters the cell as the non-ionic 1:2 chelate and then dissociates to the toxic ionic 1:1 chelate. The reason for the fungitoxicity of the 1:1 chelate within the cell is unknown. Albert et al (2) proposed that the 1:1 chelate combines with and blocks metal-binding sites on vital bacterial enzymes. Block (4) and Horsfall (6) presented the idea that naturally occurring chelators are poisoned by taking metal from copper oxinate, and that the oxine thus freed takes, or combines with, prosthetic metals from enzyme systems.

The key to how copper oxinate can yield its copper to cellular components lies in the obvious importance of the half chelate, Cu^+Ox, to the toxicity of this compound. We propose the following explanation, based on the relative stability constants (7) of the various systems involved and the relative concentrations within the cell of the component parts of these systems.

The stability constant for the system $[CuOx_2] \rightleftharpoons [Cu^{++}][Ox^-]$ is 29, but the stability constant for the system $[Cu^+Ox] \rightleftharpoons [Cu^{++}][Ox^-]$ is only 15. The stability constant for the system $[Cu\ Histidine_2] \rightleftharpoons [Cu^{++}][Histidine^-]$, however, is 18.3, or 1,000 times as great as that of $[Cu^+Ox] \rightleftharpoons [Cu^{++}][Ox^-]$. Hence, the relatively large amounts of cellular amino acids, such as histidine, would take copper from the small amount of the half chelate, Cu^+Ox, within the cell. The amino acids could not take copper from the much more stable, fully chelated $CuOx_2$ unless the $CuOx_2$ first dissociated to Cu^+Ox. The taking of copper from Cu^+Ox by the amino acids would simultaneously release small amounts of oxine within the cell. This reaction is as follows: $Cu^+Ox + 2\ Histidine \rightleftharpoons Cu\ Histidine_2 + Ox^-$.

This reaction will produce 2 deleterious effects: 1) there would be copper "poisoning" of amino acids, proteins, and enzymes, and 2) the small amounts of oxine freed *in situ* would form stable, lipid-soluble, fully chelated complexes with cellular trace metals, such as Fe^{++}, Zn^{++}, and Co^{++}, taking them from systems that need them for biological activity. This last reaction would not only inactivate the metals by binding their reactive sites but would truly sequester the metals by removing them from the aqueous phase and taking them into the lipid or nonpolar phase of the cell.

These experiments further support the proposal that the most fungitoxic form of oxine is the copper chelate. They also provide a basis for further determination of the precise fungitoxic mechanisms involved in chelation.—Department of Plant Pathology, Univer-

Table 3.—Effect of dithizone and versene on the toxicity of oxine to spores of Aspergillus niger.

Material added (ppm)	Spore germination at various concentrations (ppm) of oxine[a]				
	0	0.062	0.25	1.0	4.0
None	+	0	0	0	0
Dithizone (100)	+	+	+	0	0
Dithizone (400)	+	+	+	+	+
Versene (100)[b]	+	+	+	0	0
Versene (400)	+	+	+	0	0

[a] + = spores germinated; 0 = spores not germinated.
[b] Tetrasodium salt of ethylenediaminetetraacetic acid.

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LITERATURE CITED

1. Albert, A. 1952. Quantitative studies of the avidity of naturally occurring substances for trace metals. 2. Amino acids having three ionizing groups. Biochem. J. 50:690-697.

2. Albert, A., M. I. Gibson, and S. D. Rubbo. 1953. The influence of chemical constitution on antibacterial activity. Part VI. The bactericidal action of 8-hydroxyquinoline (oxine). Brit. J. Exptl. Pathol. 34:119-130.

3. Anderson, B. I., and R. J. Swaby. 1951. Factors influencing the fungistatic action of 8-hydroxyquinoline (oxine) and its metal complexes. Australian J. Sci. Research Ser. B. 4:275-282.

4. Block, S. S. 1955. Fungitoxicity of the 8-quinolinols. J. Agr. Food Chem. 3:229-234.

5. Byrde, R. J. W., and D. Woodcock. 1957. Effect of the interaction between chelating agents on their fungitoxicity. Nature 179:539.

6. Horsfall, J. G. 1956. Principles of fungicidal action. Chronica Botanica Co., Waltham, Mass. 280 p.

7. Martell, A. E., and M. Calvin. 1952. Chemistry of the metal chelate compounds. Prentice-Hall, Inc., N. Y. 613 p.

8. Mason, C. L. 1948. A study of the fungicidal action of 8-quinolinol and some of its derivatives. Phytopathology 38:740-751.

9. Sijpesteijn, A. K., M. J. Janssen, and H. M. Dekhuyzen. 1957. Effect of copper and chelating agents on growth inhibition of *Aspergillus niger* by 8-hydroxyquinoline and pyridine-N-oxide-2-thiol. Nature 180:505-506.

10. Zentmyer, G. A. 1943. Mechanism of action of 8-hydroxyquinoline. Phytopathology 33:1121.

11. Zentmyer, G. A. 1944. Inhibition of metal catalysis as a fungistatic mechanism. Science 100:294-295.

12. Zentmyer, G. A., and S. Rich. 1956. Reversal of fungitoxicity of 8-quinolinol and copper-8-quinolinolate by other chelators. Phytopathology 46:33.

FACTORS AFFECTING FIELD SPREAD OF POTATO VIRUS Y IN SOUTH FLORIDA

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SUMMARY

Primary spread of potato virus Y (PVY) from infected nightshade (*Solanum nigrum* L.) to pepper (*Capsicum annuum* L., var. California Wonder) was reduced 50% by bordering plots on 3 sides with 1 row of sunflower, 70% by planting a 50-ft swath of beans outside the sunflowers, and 85% by spraying the beans weekly with parathion. After 6 weeks, disease incidence was almost the same where only sunflowers were used as in the unprotected plots. Plots surrounded by unsprayed beans plus sunflowers had about ⅔ as much disease as unprotected areas while the plots surrounded by the sprayed beans had only ½ as much disease as the unprotected treatment. Primary spread to California Wonder pepper was reduced sharply when nightshade infected with PVY was sprayed twice weekly with parathion. Demeton appeared less effective. In the greenhouse Italian El pepper was highly resistant to inoculation with PVY, either by green peach aphids or by rubbing. However, once plants of this variety were infected, titer of virus rose as high as in the susceptible variety California Wonder. In field-plot trials, spread of PVY was much slower in Italian El than California Wonder. When they were interplanted rate of spread in California Wonder was slowed while rate of spread in Italian El was accelerated.

In previous studies (2) primary spread of PVY has been reduced by planting nonsusceptible crops between virus-infected weeds and susceptible crops such as pepper. Most of those studies used 1–2 rows of sunflower (*Helianthus annuus* L.) as a barrier although a low-growing crop, collards (*Brassica oleraca* L. var. *acephala*) was tested and proved effective in one case (3). The effect of using insecticides on the plant barrier or weed area had received only limited attention and it was felt further trials would be worthwhile. This report includes the results of these additional studies as well as an attempt to evaluate the practical application of these findings in terms of effect on both primary and secondary spread of virus.

Materials and methods.—All work was done with potato virus Y (pepper vein-banding mosaic virus) (2) hereinafter called PVY. Plots were laid out in the area used for previous PVY studies. The land was level, with no trees or buildings within ½ mile of the plot area. Cultural practices were patterned after those recommended by the Everglades Experiment Station for commercial production of peppers.

California Wonder pepper was the test crop plant unless otherwise noted. Rows were 36 in. apart and seeds were planted in the field with a 1-row planter. Nightshade was inoculated in the greenhouse by rubbing and then transplanted to the experimental area for use as virus inoculum several weeks prior to planting peppers or barrier crops. Thus the weeds were well established before the experiments began. Volunteer nightshade within ¼ mile of the test area was rogued before the crops were planted.

Primary spread.—*Parathion applied to the barrier crop.*—Earlier results (2) in which demeton was applied to 1 row of sunflowers had not indicated that the chemical reduced virus spread significantly. It seemed likely, however, that with a barrier crop planted over a larger area an insecticide might result in less transmission than that obtained with a barrier crop alone. Spray experiments were designed to test this hypothesis. The sprays were applied with a 1-row small-plot sprayer (1). Three Meyer's Jumbo Nozzles with No. 3 orifices were used to obtain maximum coverage. Pressure was held at 250 lb/sq in. Parathion

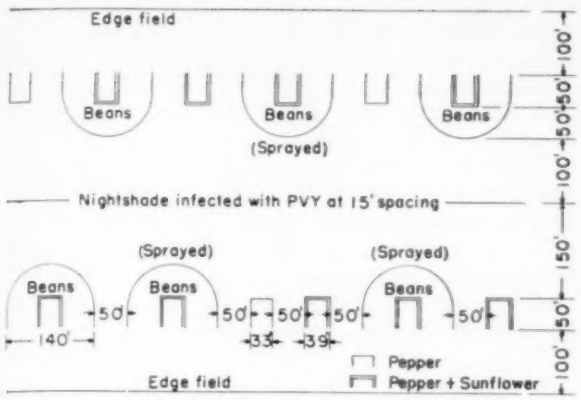

Fig. 1.—Field-plot diagram for experiment designed to show effect of barrier plantings and insecticides applied to the barriers on primary spread of PVY. Only the beans were sprayed with parathion.

(*O,O*-diethyl-*O*-*p*-nitrophenyl thiophosphate) was applied as a 15% wettable powder, and demeton (*O,O*-diethyl-*O*(and *S*)-(2-ethylthioethyl) phosphorothicates) as a 26.2% emulsion concentrate.

Twelve pepper plantings of 15 rows each, 3 ft apart and 50 ft long, were made on February 1, 1957, in a field of 600 x 830 ft. These were laid off lengthwise in 2 groups of 6 each (Fig. 1) and bordered on 3 sides by the following types of barriers: 1) 1 row of sunflowers; 2) 1 row of sunflowers plus 17 rows of snap beans (*Phaseolus vulgaris* L., var. Tendergreen) planted in rows 3 ft. apart; or 3) left unprotected. Three plots were left unprotected and 3 were surrounded by 1 row of sunflower. Six of the plots were surrounded by sunflowers and beans, of which 3 were sprayed weekly with parathion at a concentration of 2 lbs per 100 gal. Thus 3 replications of each of the 4 treatments were provided (Fig. 1). The barrier crops were planted on February 8, 1957. Nightshade plants infected with PVY were planted lengthwise down the center of the field at 15-ft intervals to serve as inoculum for naturally occurring aphid vectors. The peppers were thinned to 15-in. spacings early in March.

Virus disease readings on all plants were first taken on April 22 and continued at weekly intervals until June 3 (Table 1). The most effective barrier in preventing primary spread was the sunflower plus sprayed beans, with the sunflower alone providing the least protection. On April 22 virus incidence in the plots surrounded by sunflowers and sprayed beans was less than one-sixth that of the unsprayed check, and 6 weeks later it was still only half that in the control plots. As before (2), sunflowers alone gave initial protection but after a few weeks this effect was largely lost. The sunflower plus unsprayed bean treatment was intermediate in effectiveness, both in preventing introduction of virus and also in terms of late season control. It is apparent that a 50-ft band of beans gave much better protection than did a single row of sunflowers.

Table 1.—Effect of plant barrier crops, with and without parathion, on incidence of PVY in pepper. Parathion was sprayed weekly on the beans.

Treatment	% disease on several dates			
	4/22	4/29	5/27	6/3
Unprotected	1.3	2.9	24.1	49.7
1 row sunflower	0.6	0.7	21.2	43.7
1 row sunflower + 50 ft beans (unsprayed)	0.4	1.0	11.1	33.5
Sunflower + 50 ft beans sprayed with parathion	0.2	0.8	11.2	25.5

Spraying PVY-infected weeds with parathion and demeton.—Little is known of how insecticides applied to weed areas around fields affect the introduction of nonpersistent aphid-borne viruses from these areas. Simons reported (2) that primary spread of PVY from nightshade growing within 10 ft. of pepper was reduced by applying parathion to the nightshade. Whether this effect would be more pronounced if the nightshade plants were more distant from the crop and whether a systemic aphicide would be more effective than a contact chemical were questions that needed answering.

The experimental layout shown in Fig. 2 involved 2 sets of nightshade circles with respective diameters of about 35, 75, 125, and 225 ft. Each was separated from the others by at least 100 ft of clean-cultivated land. Inoculated nightshade plants were set out at 10-ft intervals along the circumference of each circle, making the density of inoculum plants constant. Before being used experimentally they were allowed to grow for about 6 weeks, by which time each plant was nearly 4 ft in diameter and 2.5 ft high.

Since primary spread was being checked, the technique of using potted pepper plants for 5-day exposures was used (2). Pepper plants in the 6–8-leaf stage and growing in 4-in. clay pots were transplanted in the pots in concentric circles 4 ft apart within the large nightshade circles. Each nightshade circle received 55 pepper plants placed in the following manner: 1 plant in the center; 9 plants in first circle; 18 plants in second circle; and 27 plants in the outer-

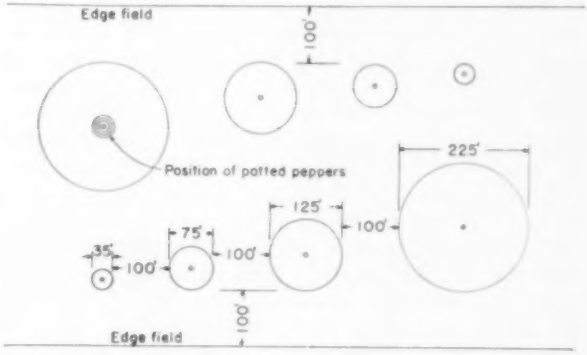

Fig. 2.—Diagram of plot design for experiment designed to test effectiveness of spraying insecticides on primary spread of PVY from nightshade to pepper. PVY-infected nightshade plants were placed 10 ft apart in each circle. Space between circular rows of peppers (upper left is an example) was 4 ft.

Table 2.—Effect on primary spread of PVY from diseased nightshade to pepper as a result of spraying the nightshade with parathion. Pepper plants were exposed to vectors for 5 days only. Parathion was applied twice during this period.

Treatment	% transmission when distance from source was:			
	12 ft.	32 ft	62 ft	100 ft
Unsprayed	36*	14	7	5
Sprayed	9	4	4	3

* Based on an average of 3 trials using 55 plants for each treatment in each trial.

most circle. These plants were spaced about 3 ft apart in each circle. The plants were set out early on a Monday morning and brought back to the greenhouse on the following Friday afternoon, where they were held for symptom development. One nightshade circle of each diameter was selected for treatment with insecticide, and one circle of each diameter was left as a control. In each week the experiment was repeated (3 times in all) the positions of the sprayed circles were rerandomized. Parathion was applied at a concentration of 3 lb of the formulation per 100 gal. to the nightshade circles on Monday and Wednesday mornings. The combined results are shown in Table 2.

Spraying with parathion had a decided effect on primary spread, whether the host weeds averaged 12 ft or 110 ft from the susceptible crop. The degree of effectiveness was greater at the close distance (4:1 reduction) than at longer distances (1.8:1 reduction). It appeared likely that some transmission resulted from contaminating infections caused by aphid flights from unsprayed nightshade circles to pepper plants in the sprayed areas. The bias would be especially great in the treatments (larger-diameter circles) where infection was lowest. To assess the degree of such spread a replicated experiment was carried out in which a PVY diseased pepper plant was surrounded by 3 concentric circles of healthy potted peppers, 4, 8, and 12 feet from the source of infection (54 plants in total), and the amount of transmission in these circles was determined during a 5-day period. After 5 days, the percentage of infection at these distances was 32, 21, and 13, respectively. Extrapolation of the gradient of infection that appeared suggests that about 1.5–3% infection would result from this type of flight at distances of 50–100 ft from an unsprayed source in a 5-day period. It is interesting to note that the earlier work on this problem (2, Fig. 2-B) indicates 4–6% transmission in 6 days' exposure at 50–100 ft from the inoculum source. Thus, the effectiveness of parathion sprays in preventing virus introduction in the larger circles was probably greater than appears from the data in Table 2.

The use of 2 heavy doses of parathion per week around a field would be very expensive for most growers. It seemed advisable to investigate the efficacy of a systemic aphicide under conditions similar to those used above. Demeton was chosen for testing. Because potted peppers were scarce, only the smaller 2 pairs of circles were used. The other circles were sprayed with 2,4-D and 2,4,5-T weed killer and destroyed. One each of the 35-ft and 75-ft circles of nightshade was sprayed with demeton at a concentration of 2 pt per 100 gal. on a Monday morning. Fifty-five peppers were set out in each of the 4 circles the same morning and left until Friday afternoon. During each of the next two weeks the procedure was repeated except that no more demeton was applied. Thus it was possible to get an estimate of the effectiveness of demeton over a period of 3 weeks. Results are shown in Table 3.

During the first week virus spread was very heavy in the unsprayed circles, with 76% of the plants contracting the disease. Demeton reduced the spread to about $\frac{2}{3}$ of what it was in the check areas. It did not seem as effective as the parathion had been in the earlier test. Residual action in terms of virus control was not detectable during the second or third week. Aphids did not reproduce on the sprayed nightshade but the chemical had no effect on virus spread. It seems likely that the contact action of parathion is important in explaining its effectiveness. Demeton has contact action for a short time after it is applied but is rapidly taken up by the plant.

Secondary spread.—*Resistance to infection with PVY of several pepper varieties.*—The preceding studies were concerned almost entirely with practices that affect primary spread of virus. Yet the evidence is abundant that most of the total spread in the field results from within-field flights of aphids. Since attempts to control vectors by insecticides in the crop have been ineffective in preventing transmission (2), the only other obvious approach was to find pepper varieties that either had resistance to infection or were poor inoculum sources of virus to the aphids.

As a preliminary experiment, 8 varieties of pepper were tested for resistance under greenhouse conditions to infection by single, apterous, green peach aphid inoculation with PVY. Aphids were reared on California Wonder pepper and starved for 1–2 hours before being given acquisition feedings of 15–20 seconds on a diseased California Wonder pepper plant.

Table 3.—Effect of demeton on primary spread of PVY to pepper when applied to diseased nightshade. Pepper plants were exposed to vectors for 5 days only.

Weeks after spraying	Treatment	% transmission when av. distance from infected nightshade was	
		12 ft	32 ft
1	Unsprayed	76*	34
	Sprayed	42**	20
2	Unsprayed	72	57
	Sprayed	55	77
3	Unsprayed	35	12
	Sprayed	29	14

* Based on a 55-plant sample.
** $\chi^2 = 8.6$, $p = < .01$.

Table 4.—Comparison of susceptibility to inoculation of PVY by single green peach aphids from infected California Wonder pepper to 8 varieties of pepper.

Pepper variety	% transmission	Expected
Anaheim Chili Hot	53[a]	
California Wonder	45	70
Early Cal Wonder	70	
Florida Giant	58	
Hungarian Yellow Wax	45	
Italian El	15**	
Red Cayenne	65	
Yolo Wonder	48	

[a] Based on a sample of 40 plants.
** $\chi^2 = 8.0$, $P = < .01$ when compared to California Wonder.

They were then transferred singly to 10 healthy pepper plants of each variety in the 2–4-leaf stage of growth. The experiment was repeated 4 times so that 40 plants of each variety were tested. Italian El pepper was much more resistant to infection than all other varieties (Table 4), which were not significantly different from California Wonder.

The reduced susceptibility of Italian El pepper possibly would not have appeared if Italian El had been used as the inoculum source variety. Therefore, a factorial experiment was carried out in which California Wonder and Italian El varieties were compared as both virus inoculum sources and test plants. Green peach aphids were treated as before and 1 aphid was used per test plant. The inoculum plants had been infected for about 2 weeks. Twenty plants of each variety were inoculated from each source in 2 trials so that 40 plants were inoculated for each of the 4 treatments. Results were as follows: 1) California Wonder used as the inoculum source gave 60% transmission to California Wonder and 10% infection to Italian El. 2) Italian El as the inoculum source resulted in 70% transmission to California Wonder and 8% to Italian El.

The varieties were not significantly different as sources of inoculum to California Wonder and to Italian El (χ^2 = approx. .30, $P = > .80$). Regardless of the inoculum variety used, susceptibility was high for California Wonder and low for Italian El. The difference in susceptibility between the 2 varieties was highly significant ($\chi^2 = 16.2$, $P = < .01$).

Since previous work (4) had shown a high degree of correlation between amounts of PVY transmission obtained by using single green peach aphid inoculations from California Wonder and rubbing of California Wonder pepper sap diluted 1:500, it was decided to compare susceptibilities of the 2 varieties in this manner. California Wonder pepper plants infected for 2 weeks were used for inoculum. After using a plant for an aphid inoculum source, its leaves were ground with a mortar and pestle and the sap (diluted 1:500 in tap water) was used for rubbing trials. California Wonder and Italian El varieties in the 2–5-leaf stage were used as test plants. Twenty plants of each variety were inoculated by aphids and 25 of each variety by rubbing. The experiment was repeated once, so that in all 40 plants for each treatment were inoculated by aphids and 50 by rubbing. Results were as follows: 1) Aphid inoculation to California Wonder gave 50% infection, whereas rubbing gave 68%; 2) Aphid inoculation to Italian El resulted in 10% transmission, whereas rubbing caused 8% infection. Both varieties responded similarly to either type of inoculation. As before, California Wonder was highly susceptible and Italian El highly resistant to infection.

Italian El pepper is highly resistant to infection with PVY, but plants of this variety that do become diseased are similar as sources of virus to those of the highly susceptible California Wonder variety. Evidently the same barrier against infection exists to both aphid and rubbing types of inoculation in both varieties of pepper.

Rate of spread of PVY in field-grown California Wonder and Italian El varieties of pepper.—It seemed likely that differences in susceptibility of the magnitude found between California Wonder and Italian El peppers would be reflected in relative rate of primary spread in the field. Since titer of virus was similar in both varieties once the plants were infected, the effect on secondary spread would probably be less pronounced. The following experiment was designed to test these ideas.

Sixteen plots of 33×33 ft were laid out in a 4×4 Latin Square arrangement. Each was separated from the other by 50 ft of clean-cultivated ground. Plots were planted in September with 12 rows spaced 3 ft apart in the following arrangements: 1) seeded entirely to California Wonder variety, 2) seeded entirely to Italian El variety, 3) seeded every other row with each variety, and 4) seeded 3 rows alternately with each variety. PVY-infected nightshade planted in the area for inoculum purposes was eaten by rabbits before the plants could grow. Thus virus was introduced into the plots from sources at least 150 ft away. Disease readings were made at 7–11-day intervals beginning on December 1, when the first virus symptoms appeared. A killing frost on January 3 terminated the experiment. Results are shown in Table 5.

Table 5.—Results of interplanting experiment on field spread of PVY in a susceptible variety (California Wonder) and a resistant variety (Italian El). Plots were arranged in a 4×4 Latin Square.

Treatment	% infection on several dates			
	Dec. 1	Dec. 8	Dec. 18	Dec. 29
All California Wonder	0.2[a]	1.1	2.7	12.1
All Italian El	0.0[a]	0.1	0.1	0.4
Every other row				
(California Wonder	0.4[b]	1.6	4.4	10.2
and Italian El)	0.0[b]	0.0	0.2	3.2
Alternate 3 rows				
(California Wonder	0.2[b]	0.6	1.6	5.2
and Italian El)	0.0[b]	0.0	0.4	1.6

[a] Based on 1000 plants.
[b] Based on 500 plants.

Table 6.—Effect of using 1, 3, and 6 green peach aphids per test plant on transmission of PVY to Italian El pepper. California Wonder pepper was used for virus inoculum.

No. aphids per test plant	Transmission	
	Observed	Expected[a]
1	1/30	2/30
3	6/30	6/30
6	8/30	10/30

[a] Based on the assumption that infections are local and independent. If p = probability of infection by 1 insect, the probability of noninfection (q) is 1–p; thus p = 1–q. To determine the probability (P) of transmission by x insects, $P = 1-q^x$.

Although virus was found in the Italian El plots within a week of its appearance in the California Wonder plots, at the end of the experiment there was 30 times as much PVY in the susceptible variety. There seems little doubt that relative susceptibility of the 2 varieties greatly influenced the rates of primary spread of PVY.

The pattern of total (primary plus secondary) spread was different when the 2 varieties were interplanted. The presence of the resistant variety tended to reduce infection in the susceptible, particularly where the 3-row groups were used. Rate of spread in Italian El variety was markedly higher when it was planted next to California Wonder. The inoculum furnished by diseased California Wonder plants in these plots caused this.

These results suggested that the resistance of Italian El pepper to infection with PVY would be broken down if enough viruliferous aphids fed on the plants. Since it is generally accepted that the chances for infection are local and independent (6), a trial was set up to test this point under greenhouse conditions. Italian El plants were inoculated by means of 1, 3, and 6 green peach aphids per plant. Duplicate trials (15 plants for each of the 3 vector groups) were carried out. Results (Table 6) show that the amount of infection obtained was similar to that expected if each aphid acted independently as a vector, thus confirming what had been observed in the field.

Discussion.—Sylvester and Simons (5) reported on a transmission phenomenon involving *Brassica nigra* virus in two plant species and the green peach aphid that seems similar to that described here for the two varieties of pepper and the green peach aphid. One of these plants, *Brassica juncea* Coss (mustard), was much more susceptible to infection by green peach aphid inoculation than the other, *B. chinensis* L. (pak choi). Both were equally potent as inoculum source plants for green peach aphids. Because the false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis), was more efficient than the green peach aphid at transmitting virus to pak choi, it was hypothesized that the differences were the result of a specific compatibility between the host plant and the vector. The results obtained here with PVY do not disprove this, but the similarity in results of the comparative inoculation trials (Table 6) strongly suggests that differences in host species have more influence than differences between vector species on chances of obtaining transmission in such a situation.—Everglades Experiment Station, University of Florida, Belle Glade.

LITERATURE CITED

1. Cox, R. S., D. S. Harrison, and C. S. Yager. 1955. A versatile spray rig for small field plots. U. S. Dept. Agr. Plant Disease Reptr. 39:48-50.
2. Simons, J. N. 1957. Effects of insecticides and physical barriers on field spread of pepper veinbanding mosaic virus. Phytopathology 47:139-145.
3. Simons, J. N. 1958. The effect of movements of winged aphids on transmission of a nonpersistent aphid-borne virus. Proc. Tenth Intern. Congr. Entomol. 3:229-231.
4. Simons, J. N. 1958. Titers of three nonpersistent aphid-borne viruses affecting pepper in south Florida. Phytopathology 48:265-268.
5. Sylvester, E. S., and J. N. Simons. 1951. Relation of plant species inoculated to efficiency of aphids in the transmission of Brassica nigra virus. Phytopathology 41: 908-910.
6. Watson, M. A. 1936. Factors affecting the amount of infection obtained by aphis transmission of the virus Hy. III. Phil. Trans. Roy. Soc. London Ser. B. 226:458-489.

SEROLOGICAL STUDIES OF COMMERCIALLY PRODUCED PLANT-VIRUS ANTISERA

Committee on Virus Type Culture Collection, American Phytopathological Society

Introduction.—The Committee on Virus Type Culture Collection started a project to 1) explore the possibilities of commercial production of plant virus antisera from purified antigens supplied from several distant sources, and 2) stimulate interest in the use of serological methods in the study of plant viruses.

Although the value of these methods as an aid in virus identification and the screening of vegetatively propagated plants to select virus-free material has become increasingly evident over the years, most plant pathologists have been reluctant to adopt the methods of serology. Fortunately, however, this situation is changing. A few of our plant pathology laboratories are using animals, and several of our virus pathologists have spent time in these laboratories or in foreign (Netherlands) laboratories to obtain a working knowledge of serological methods.

The present studies show that good antisera were produced by all purified antigens shipped from several distant laboratories to where the animal work was done: Microbiological Associates (M.A.), Bethesda, Maryland, a company engaged in producing sera, animal tissue-culture media and cell lines, and related products. Some financial problems in connection with the profitable commercial production of plant-virus antisera, however, have not been solved. It seems apparent now that it may be too expensive to produce only 1 or 2 antisera at a time on the order of individ-

ual investigators prepared to supply the antigens. It appears that investigators should pool their needs. This may be done through the Committee, and when there is a demand for a sufficient number of antisera, arrangements can be made for their commercial production from the antigens to be supplied.

Methods and results.—The purity of antiserum depends on the purity of the antigen used for its preparation. Two types of purity are important when a plant virus is used as an antigen: 1) freedom from other viruses, and 2) freedom from antigens normal to healthy plants. It is impossible to guarantee that any preparation of virus is absolutely pure in these two respects, because if available tests for detection of contaminants are negative, it means that their concentration is below a certain limit, not necessarily that they are completely absent. Most of the viruses used for the present program are in the American Collection (See Catalogue and Registry of the Plant Viruses, 2nd Edition, American Type Culture Collection, Washington 7, D. C.). The corresponding antisera are represented as antisera specific for these particular cultures of viruses, and the Committee makes no further guarantee as to their purity. For convenience, the antisera are referred to herein by the common names of the viruses.

As regards the presence of antibodies specific for antigens normal to healthy plants, no claim is made that they are completely absent. Purified viruses were used for injecting the rabbits, however, and the antisera give little or no reaction with extracts of healthy plants. None of the antisera have been absorbed with extracts of healthy plants.

The viruses included in this trial program were selected on the basis of ease of purification, stability, and potential demand for the antisera. The viruses were purified by cooperating plant pathologists and shipped to M.A., where the antisera were produced. The sera were titrated and recommendations for pooling made by Ellen L. Moorhead. In addition, some of the antisera were titrated by the contributors of the corresponding viruses.

Barley-stripe mosaic, brome mosaic, potato latent (X-virus), southern bean mosaic, and tobacco mosaic viruses were purified by 2 or more cycles of differential centrifugation. Either the diseased leaves or the extracts therefrom were frozen before the centrifugation. In addition, the extract containing tobacco mosaic virus was heated to 45°C and clarified by filtration through Celite before centrifugation. Cymbidium mosaic virus was purified from an extract of frozen leaves by differential centrifugation, density-gradient centrifugation, and zone electrophoresis in density-gradient columns. Tobacco ringspot and alfalfa mosaic viruses were purified by Steere's (5) method of differential centrifugation of an extract treated with n-butanol and chloroform.

Several rabbits were injected with each virus. After emulsification in mineral oil by the method of Salk and Laurent (4), the viruses were injected intramuscularly. Standard techniques were used to obtain and process the sera. The sera from individual bleedings were titrated by the complement fixation test under the conditions specified by Moorhead (2). Sera with the highest titer for each virus were pooled, and more extensive tests made on the pooled sera.

Table 1 lists the viruses, their American Culture

Table 1.—Comparative data from serological tests of plant virus antisera prepared by M.A.

Virus	American[a] Collection number	Reciprocal of the dilution endpoints that contain the optimum reacting units of antigen (Ag) and of antiserum (Ab) as determined by various serological tests								Contributor of antigen
		Complement Fixation		Sheep red blood cell precipitin		Micro-precipitin		Ring interface precipitin		
		Ag	Ab	Ag	Ab	Ag	Ab	Ag	Ab	
Alfalfa mosaic		4096[b]	64	1024	8	512	16			R. Steere
Barley stripe mosaic	AC-69	128	64	32	16	32	128 (256)[c]	32	256 (64)	M. K. Brakke
Brome mosaic	AC-66	4096	256	1024	32	2048	128 (64)	1024	256 (256)	M. K. Brakke
Cymbidium mosaic		1024	512	128	64	64	256	256	512	H. Gold
Potato latent (X-virus)	AC-61	32	512			256	256	1024	1024	A. F. Ross
Southern bean mosaic	AC-18	2048	256	256	128	512	256			M. K. Brakke
Tobacco mosaic	AC-2	8192	256	512	64	256	256 (256)	256	256	H. H. Thornberry and G. Silber
Tobacco ringspot		1024	512			64	512			R. Steere

[a] American Collection numbers assigned to cultures listed in the Catalogue and Registry of Plant Viruses, 2nd Edition, American Type Culture Collection.

[b] All titers are based on grid titrations. Endpoints represent the highest dilution of antigen that reacts with the least amount of serum to give a specified positive result.

[c] Test results in parentheses were obtained by contributors, and do not necessarily represent the same endpoints as described above.

(AC-) numbers, the contributors of the purified viruses, and a summary of the serological tests of the pooled sera. Purified viruses were used to titrate the antisera in the serological tests; in most cases, enough antigen was prepared that samples from the original solution were used for immunization and for serological tests. The antigen dilutions are based on an arbitrary concentration of virus, but this remained the same for all tests on the same antigen-antibody system. Comparative data were obtained from complement fixation, sheep red blood cell precipitin (3), micro-precipitin (6), and ring interface precipitin tests. From grid titrations, an attempt was made to pick similar serological endpoints in all the test techniques. These endpoints were contained in the mixture representing the highest dilution of antigen giving a positive reaction with the least amount of serum. The data in Table 1 show that the complement fixation test was more sensitive in demonstrating antigen-antibody combination, except where aggregation of the purified virus was a problem, e.g., potato latent and barley stripe mosaic viruses. The micro-precipitin test had the distinct advantage of being easier to set up, but the interface test was the more sensitive of the precipitin tests. The sheep red blood cell test could not be used with potato latent virus, because of spontaneous precipitation of the antigen. All these data illustrate the fact that the serological endpoints from an antigen-antibody system depend on the type of test used. The endpoints obtained with the tests vary with the virus, with the conditions of the test (time of incubation, criterion of positive reaction, lighting used to read the tests, etc.), and with the individual observer. None of the titers presented in the report can be considered absolute. Anyone using these antisera should use the data as a guide, but they should test the sera against corresponding viruses to determine the endpoints under their own specific conditions. Ideally, a sample of the American Collection culture of virus used in the production of the antisera should be used to test the sera. For identification of an unknown virus, antisera should be reacted with extracts from uninfected plants, plants infected with the American Collection culture, and plants infected with the unknown virus. The degree of purification of the extracts will vary with the virus and the serological test, but all extracts must be treated alike. Finally, a control with normal serum should be run.

Table 2.—Plant viruses for which specific antisera are presently being used in the United States and Canada.

Researcher	Virus
A. H. Gold Plant Pathology Department University of California Berkeley 4, California	Barley stripe mosaic Bean yellow mosaic (broadbean mosaic strain) Clover, White clover mosaic Melon veinal necrosis Potato latent (X-virus) Potato S Sugarcane mosaic, Calfornia strain Sugarcane mosaic, U.S.D.A., Db strain Tobacco mosaic Tobacco necrosis Tobacco rattle, potato-infecting strain
Ellen L. Moorhead U. S. Dept. of Agriculture Plant Pathology Department University of Nebraska Lincoln, Nebraska	Alfalfa mosaic Barley stripe mosaic, AC-69 Bean, Southern bean mosaic Brome mosaic, AC-66 Cucumber green-mottle mosaic Potato latent (X-virus) Potato S Tobacco mosaic (15 strains) Tobacco necrosis Tobacco ringspot Wheat streak mosaic, AC-29
John B. Bancroft Dept. of Botany & Plant Pathology Purdue University Lafayette, Indiana	Alfalfa mosaic Bean pod-mottle Clover, Red clover vein mosaic
Dr. R. J. Shepherd Plant Pathology Department University of Wisconsin Madison, Wisconsin	Bean, Southern bean mosaic, AC-17 Cowpea mosaic Cucumber mosaic, AC-127 Potato latent (X-virus) Squash mosaic Tobacco mosaic Tobacco necrosis, AC-39 Tobacco ringspot Turnip mosaic Turnip yellow mosaic Wild cucumber mosaic
N. S. Wright Science Service Plant Pathology Laboratory c/o University of British Columbia Vancouver 8, B. C., Canada	Potato latent (X-virus) (12 strains) Potato S Tobacco mosaic (2 strains)
R. H. Bagnall Canada Department of Agriculture Research Station P. O. Box 280 Fredericton, N. B., Canada	Carnation latent Potato F Potato latent (X-virus) Potato M Potato S
L. M. Black and collaborators Department of Botany University of Illinois Urbana, Illinois	Potato yellow-dwarf Tomato spotted-wilt Wound-tumor

In addition to the tests listed in Table 1, antigens made from clarified extracts of diseased plants were also used to test the activity of the sera. All of the antisera gave positive micro-precipitin results with homologous antigens. Cymbidium mosaic virus antiserum was not tested, since infected plant material was not available. None of the antisera gave positive reactions with extracts from uninfected plants. In addition, all antisera gave positive reactions in the agar diffusion test of Gispen (1) with homologous purified viruses except tobacco ringspot and potato latent viruses. These presumably failed because of aggregation of the virus. A positive agar diffusion test was obtained with antiserum of potato latent

virus and with unpurified extract from diseased plants. Some of the virus evidently remained unaggregated in the unpurified extracts. None of the antisera gave cross-reactions with any other virus except the antiserum of Cymbidium mosaic virus. This reacted at a dilution of 1:4 with tobacco mosaic virus in complement fixation tests. The antiserum of tobacco mosaic virus, however, did not react with the Cymbidium mosaic virus preparation.

Samples of the antisera listed in Table 1 will be made available at the discretion of the Committee. Graduate students should have their requests approved by the Chairman of their department. Those interested in obtaining samples should write to the Chairman of the Committee, H. H. McKinney, Plant Industry Station, Beltsville, Maryland.

Several laboratories in the United States and Canada are presently producing antisera specific for plant viruses for use in their own research programs. Exchanges of samples of antisera among laboratories working on the same or similar viruses are often made to confirm identification of the viruses. Often, antisera are exchanged more readily than infectious material. To facilitate such exchanges the Committee will periodically publish a list of antisera presently being used in various laboratories. The first such list is presented in Table 2. Exchanges of samples of antisera presented in this list are entirely at the discretion of the individuals who prepared them, and neither the exchange nor the arrangement of the exchange is a function of the Committee. The Committee will welcome information on other antisera that should be included in future lists.

Institutions collaborating with the Committee in a major way: Crops Research Division, A.R.S., U. S. Department of Agriculture; Department of Plant Pathology, University of Nebraska; Microbiological Associates.

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LITERATURE CITED

1. Gispen, R. 1955. Analysis of pox-virus antigens by means of double diffusion. A method for direct serological differentiation of cowpox. J. Immunol. 74:134-141.

2. Moorhead, Ellen L. 1956. Serological studies of viruses infecting the cereal crops. I. A comparison of barley stripe mosaic virus and brome mosaic virus isolates by means of the complement-fixation technique. Phytopathology 46:498-501.

3. Moorhead, Ellen L., and W. C. Price. 1953. A new serological test for tobacco mosaic virus. Phytopathology 43:73-77.

4. Salk, J. E., and Angela M. Laurent. 1952. The use of adjuvants in studies on influenza immunization. I. Measurements in monkeys of the dimensions of antigenicity of virus-mineral oil emulsions. J. Exptl. Med. 95:429-447.

5. Steere, R. L. 1956. Purification and properties of tobacco ringspot virus. Phytopathology 46:60-69.

6. van Slogteren, D. H. M. 1955. Serological microreactions with plant viruses under paraffin oil. Proceedings of the Second Conference on Potato Virus Diseases. (1954) Institute for Phytopathological Research, Wageningen, The Netherlands. p. 51-54.

STUDIES IN THE DISEASES OF MANGIFERA INDICA
XI. THE EFFECT OF BORON ON MANGO NECROSIS

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SUMMARY

Characteristic tip necrosis of the mango fruit was reduced by boron sprayed in the form of borax. Of 3953 sprayed fruits, only 179 became necrotic; of 2843 control fruits, 898 became necrotic. Statistical analysis of the data showed that boron very significantly reduced mango necrosis, degree of air pollution affects the degree of necrosis, and efficacy of borax may be unchanged by degree of air pollution.

Introduction.—The necrosis of mango fruit in which the tissues of distal end break down, forming a well defined necrotic area characteristic of the disease, results from exposure to fumes emanating from brick-kilns operating near the orchards (2, 21). Intensive work has been carried out by the senior author and his co-workers extending for a number of years on different aspects of the problem to find out the cause of the disease and its control (1–7). While attempts to reproduce necrosis by various constituents of brick-kiln fumes including sulphur dioxide (21, 6, 23), carbon monoxide (1, 22), ethylene (22, 23), fluorine, etc. did not meet with success, in recent years the causal agent has been shown to be a chemical substance isolated from both fumes and diseased mangoes (6, 7).

Proceeding on the assumption that the disturbance of metabolic activity of the fruit induced by the causal agent resulting in the tip necrosis can be of the nature

Table 1.—Effect of borax sprays on the development of necrosis in mango fruit.

Plot	Treatment[a]	Sprayed trees: Total number fruits	Sprayed trees: % fruits with necrosis	Unsprayed trees: Total number fruits	Unsprayed trees: % fruits with necrosis
Comparison of plots:					
A	6-B once	1259	1.90	1115	16.41
	6-B twice	594	1.68		
B	6-B once	40	15.00	313	24.92
	8-B once	972	3.40		
C	8-B once	1118	9.48	343	44.02
D				1082	43.07
Total		3983	4.49	2853	30.77
Comparison of sprays:					
	8-B once	2090	6.65	656	34.91
	6-B once	1299	2.36	1428	18.28
	6-B twice	594	1.68	1115	16.41

[a] 6-B is 6 lb borax; 8-B is 8 lb borax.

of a mineral deficiency some preliminary trace element studies were made. The information thus obtained appeared to indicate that the disturbance in boron metabolism leading to its deficiency in the actively growing region of the mango fruit might be the key to the problem. The spraying of boron as a method of controlling necrosis was then undertaken the results of which are presented in this paper.

Materials and methods.—The spraying experiments were carried out in an orchard situated in the outskirts of Lucknow. This orchard had 5 operating brick-kilns within a radius of about ½ mile: 2 on the east and 1 each in the other three directions. All the important varieties of mangoes in the orchard—Dasheri, Safeda, Langra, and Fazli—were known to suffer every year from some necrosis. The experiments were restricted to Dasheri because this variety consistently showed the highest incidence of disease.

In spraying, the method of Pyenson (17) was followed. The trees were first sprayed from a position near the trunk, then from a position outside of the branches. The spray guns were moved at a steady speed in vertical up and down sweeps, overlapping slightly each time and then at the top and sideways for good coverage of the foliage. The spraying was continued until the foliage was wetted to slight runoff.

Sixty blossoming Dasheri trees distributed in the 4 plots of the orchard, designated as A, B, C, and D, were used for experiment. Trees in C and D, being nearer to and in the leeward direction from two kilns on the east, were known to have been more severely affected than trees in A and B.

Two concentrations of borax—6-B, 6 lb borax in 100 gal. well water; and 8-B, 8 lb borax in 100 gal.—were used for the spraying.

Treatments were given as follows: Altogether, 30 trees were treated with boron, of which 5 trees in plot A were sprayed twice with 6-B; 9 trees in plot A and 1 in plot B sprayed once with 6-B; and 15 trees, 6 in plots B and 9 in C, sprayed once with 8-B. Another 30 unsprayed trees—11 in plot A, 5 in plot B, 4 in plot C, and 10 in plot D—served as controls.

The set of 5 trees to be treated twice were sprayed first with a hand sprayer on March 25, 1956, when most of the branches were in full bloom and some branches had just completed the blossom stage. This set was treated again on March 31, when most of the branches had just completed the blossom stage. The other sets were also sprayed at this time. All 3 sets were sprayed with a power sprayer at 200 lb pressure.

The progress of the disease was observed periodically and a final fruit count was made on May 27, 57 days after the last spraying, when fruit length was about 4 in. Fruits showing a slight visible etiolation were considered diseased. The results are summarized in Table 1.

Results.—The data (Table 1) clearly indicate that incidence of necrosis was substantially reduced by boron treatment at an early stage of fruiting. With all treatments considered together, disease was 4.5% among sprayed fruits and 30.8% for the unsprayed. The percentages of the individual treatments were: 8-B—sprayed once, 6.7; unsprayed, 34.9. 6-B—sprayed once, 2.4; unsprayed, 18.3. 6-B—sprayed twice, 1.7; unsprayed, 16.4.

The greater disease in plot C (9.5%) than in plot B (3.4%) among plants receiving 8-B implies that the amount of boron required to prevent necrosis may vary with the intensity of the disease, that is, more highly affected orchards may need higher doses of boron.

It was also observed that 83.8% (150 in 179) and 87% (764 in 878) of the diseased fruits in the sprayed and unsprayed plants respectively reach the third stage of necrosis. Apparently, although a given quantity of boron prevented or checked the disease, depending on time of application, individual fruits may fail to respond to boron because of undetermined factors.

The data obtained from the experiments were evaluated by the chi-square test of independence, allowing for the percentage of healthy fruits in control trees. It was found that the percentage of necrosis in sprayed fruits was 14.59 when unsprayed fruits were 100% necrotic.

Because the fruits are classified in two fashions—1) sprayed and unsprayed, and 2) diseased and healthy—it was of interest to test whether these classifications are independent of each other.

Separately for each set of sprayed plants and the corresponding controls, it is observed statistically by studying Table 1 that since chi-square value is very high, 878, the observations are clearly opposed to the hypothesis of independence, and it can be said that there is a definite preventive effect of borax on mango necrosis.

To test whether the proportion diseased is independent of air pollution, only fruits uniform in all respects except exposure to brick-kiln fumes are considered. Therefore only the fruits of the control plants of different plots are considered. The value of chi-square, 151 (approx.), is too high for 3 degrees of freedom, and it is concluded that degree of air pollution affects the degree of necrosis.

Because 6-B was sprayed twice on some plants and

only once on others, it was of interest to test whether double spraying is superior to single spraying. Here the test plants of plot A are considered alone. All were sprayed with the same concentration of borax solution, some twice and some only once. In random sampling such deviations may occur in more than 37% of the cases. Thus, as far as this sample goes, spraying twice is in no way superior to spraying once in preventing necrosis.

Lastly, it is also possible to test from these data whether air pollution reduces the efficacy of borax treatment. Here are considered the mangoes sprayed with 8-B in plots B and C. The fruits are uniform in every respect except the air pollution factor. Under the hypothesis of no difference, the value of z, 5.57, corresponds to a probability of less than 0.001. Therefore the proportion diseased differs significantly in the two plots, and the efficacy of borax is not significantly different in the 2 plots.

Discussion.—The ability of boron to prevent necrosis as demonstrated by the foregoing results indicates that boron was deficient in the mango fruit exposed to brick-kiln fumes. The accumulation of glucose and nitrogen (1) in the necrotic mango fruits may be due to boron deficiency arising, in a manner as explained by Reed (19) for sugars and Hewitt (9) for nitrogen, by failure of resynthesis of adenosine triphosphate. Further, the resemblance of typical symptoms of mango necrosis to boron deficiency of olive (20) and pears (12), and the fact that a number of diseases have now been shown to be due to deficiency of boron (11, 15, 25), lend indirect support to this view.

Cases in which symptoms similar to tip necrosis appear in the apical parts of fruits because of fluorine injury have been recorded by McCornack (14) in peaches, Radeloff et al (18) in pears, Kotte (13) in plums, and Neger (16) in apples. Limited experiments by the authors with fluorine on mango trees *in vivo* failed to reproduce necrosis. But the result is far from conclusive and it would be interesting to find out if the chemical substance isolated from the brick-kiln fumes and necrotic tissues contains fluorine in some form. It is clear from the result presented here that, whatever the nature of the causal agent, mango necrosis can be prevented by the application of boron.

Fixation of boron resulting in its deficiency is known to occur, affected by low moisture content (10, 26), length of day (24), climatic condition (8), and many other factors, either in the soil or in some plant parts, especially in the leaves, rendering it unavailable to the growing regions of plants. It is suggested that mango necrosis is caused not by the fixation of boron in soil but by disturbance in boron metabolism through interaction of some constituent of brick-kiln fumes with certain cell metabolites of mango leaves and fruits.—Botany Department, Lucknow University, Lucknow, India.

LITERATURE CITED

1. Das-Gupta, S. N. 1940-1951. Studies on mango necrosis. Report submitted to Indian Council of Agricultural Research, New Delhi, 257 p. (Unpublished)

2. Das-Gupta, S. N., and G. S. Verma. 1939. Studies in the diseases of Mangifera indica Linn. I. Preliminary observations on the necrosis of the mango fruit with special reference to the external symptoms of the disease. Proc. Indian Acad. Sci. B. 9:13-28.

3. Das-Gupta, S. N., and G. S. Verma. 1940. Studies in the diseases of Mangifera indica Linn. II. Effect of injecting healthy mango fruits with extracts from naturally occurring necrotic mangoes. Proc. Indian Acad. Sci. B. 12:95-108.

4. Das-Gupta, S. N., G. S. Verma, and S. Sinha. 1941. Studies in the diseases of Mangifera indica Linn. III. Investigation into the effect of sulphur dioxide gas on the mango fruit. Proc. Ind. Acad. Sci. B. 13:71-83.

5. Das-Gupta, S. N., S. Sinha, and S. N. Asthana. 1944. Detailed investigation on the effect of sulphur dioxide and other gases on the mango fruit. Proc. 31st Indian Sci. Congr. III:89.

6. Das-Gupta, S. N., G. S. Verma, S. C. Agarwala, J. N. Rai, and S. N. Iyer. 1950. Necrosis of the mango fruit. Current Sci. India 19:153.

7. Das-Gupta, S. N., S. N. Iyer, and G. S. Verma. 1956. Studies in the diseases of Mangifera indica Linn. IX. Isolation of a brick-kiln fume constituent causing mango necrosis. Indian J. Agr. Sci. 26:259-266.

8. Eaton, F. M. 1944. Deficiency, toxicity and accumulation of boron in plants. J. Agr. Research 69:237-277.

9. Hewitt, E. J. 1951. The role of the mineral elements in plant nutrition. Ann. Rev. Plant Physiol. 2:25-52.

10. Hobbs, J. A., and B. R. Bertramson. 1949. Boron uptake by plants as influenced by soil moisture. Soil Sci. Soc. Am. Proc. 14:257-261.

11. Johnson, F., D. F. Allmendinger, V. L. Miller, and Dorothy Polley. 1955. Fall application of boron sprays as a control for blossom blast and twig dieback of pears. Phytopathology. 45:110-114.

12. Kienholz, J. R. 1942. Boron deficiency in pear trees. Phytopathology. 32:1082-1086.

13. Kotte, W. 1929. Rauchschaden an Steinobstfruchten. Nachbrl. deut. Pflanzenschutzdienst Berlin 9:91-92.

14. McCornack, A. A., L. C. Cochran, H. English, and C. L. Hemstreet. 1952. A black tip condition of peach fruits in California. Plant Disease Reptr. 36:99-100.

15. Middleton, J. T., B. J. Hall, R. T. Wedding, and J. B. Kendrick, Jr. 1952. Adaxial crack stem of celery. Phytopathology. 42:603-606.

16. Neger, F. W. 1919. Z. Obst-Gartenbau. 45:177.

17. Pyenson, L. L. 1951. Elements of plant protection. John Wiley & Son, New York. 538 p.

18. Radeloff, H. 1939. Untersuchung und Begutachtung von Rauchschaden. Jahresber. Hamburgischen Inst. Angew. Botan. 55:119-120.

19. Reed, H. S. 1947. A physiological study of boron deficiency in plants. Hilgardia. 17:377-411.

20. Scott, C. E., E. H. Thomas, and H. E. Thomas. 1943. Boron deficiency in the olive. Phytopathology. 33:933-942.

21. Sen, P. K. 1943. Black tip disease of the mango. Indian J. Agr. Sci. 13:300-333.

22. Sen, P. K., P. C. Mallik, and P. K. Roy. 1943. Toxic effect of gases on plants. Sci. and Culture Calcutta 9:87-88.

23. Verma, G. S. 1952. The formation of lesions by gases on mango fruits. J. Indian Botan. Soc. 31:316-341.

24. Warington, Katherine. 1933. The influence of length of day on the response of plants to boron. Ann. Botany (London) 47:429-457.

25. Wiebosch, W. A. 1948. Besputing van Vruchtbomen met Mangaan-en Boriumzouten. Mededel. Direct Tuinbouw. 11:320-323.

26. Woodbridge, C. G., A. Carney, and H. R. McLarty. 1952. A boron deficiency in pear growing in soil having an adequate boron content. Sci. Agr. (Ottawa) 32:446-442.

RELATION OF NITROGEN TO DISEASE DEVELOPMENT IN RICE SEEDLINGS INFECTED WITH HELMINTHOSPORIUM ORYZAE

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SUMMARY

Seedling blight and leafspot incited by *Helminthosporium oryzae* was compared in rice seedlings grown in 0.5*N* Hoagland's solution containing only NO_3 nitrogen and Hoagland's plus 3 levels of NH_4NO_3. Seven rice varieties and 3 fungus isolates were used. Conidial suspensions were used to inoculate seedlings 18–20 days old. Growth of seedlings was checked and plants showed symptoms of nitrogen deficiency at 105 ppm level of NO_3. NO_3 was apparently not used by rice seedlings in unaerated sand culture. Seedling blight maximum was in seedlings grown in solutions of 105 ppm. NO_3 only, and of 329 ppm with 217 NO_3 and 112 NH_4. Both seedling blight and lesion size were reduced in solutions with intermediate levels of NH_4 nitrogen, which were optimum for host development. The variety B52-2579 was extremely susceptible, whereas the other 6 varieties showed relatively little difference in reaction. Fungus isolates did not appear to differ greatly in pathogenicity. Germ tube development and spread of the mycelium in mesophyll, bundle sheath, phloem, and xylem, and the collapse of the tissues, were more rapid and extensive at deficient or high levels of NH_4 nitrogen than at intermediate levels. Both deficiency and excess of NH_4 nitrogen appeared to affect disease development similarly.

Helminthosporium blight of rice, incited by *Helminthosporium oryzae* Breda de Haan or *Bipolaris oryzae* (B. de Haan) Shoemaker, is world-wide in distribution and generally causes severe damage. Losses in stand, due to seedling blight, and reductions in yield and quality, due to culm, leaf, and kernel infection, are often high (2).

Precise knowledge is lacking on the relation of mineral nutrition to the development of this disease in rice. Occasional reports (1, 8, 9) have suggested the possibility that the supply and form of nitrogen is related to disease development. Application of nitrogenous fertilizers (9) and avoidance of excess of nitrogen (8) have been recommended as possible measures. Hashioka and Makino (5) reported that in plants of a susceptible variety, grown in sand cultures and inoculated at late tillering, the number and size of lesions decreased with increasing nitrogen level. The nitrogen range tested was 0–12 mg of $(NH_4)_2SO_4$ per liter.

Preliminary data on a study of the effect of nitrogen on disease development in rice seedlings are reported herein.

Materials and methods.—Experiments were conducted under controlled conditions in the greenhouse at 28°C. Seedlings were grown in washed quartz sand in porcelain crocks and watered with 0.5*N* Hoagland's solution adjusted to different levels of NH_4 and NO_3 nitrogen by addition of NH_4NO_3 (Table 1).

The varieties of rice used were Caloro (C.I. 1561-1), Toro (C.I. 9013), Zenith (C.I. 7787), Bluebonnet (C.I. 8322), Bluebonnet 50 (B45-2382), and Beaumont (B52-2579). (Seeds were obtained from Dr. E. M. Cralley, the Agricultural Experiment Station of Arkansas, and from the Rice Experiment Station at Beaumont, Texas.)

Three isolates of *H. oryzae* were used: cultures H2, H3 (an ultraviolet-induced albino mutant of H2), and H5. (H2 was supplied by Dr. G. E. Templeton, Arkansas Agriculture Experiment Station, Fayetteville, Arkansas; H5 was supplied by Dr. J. G. Atkins, Rice Experiment Station, Beaumont, Texas.)

Composition of 1*N* Hoagland's solution, on a g/L basis, is as follows: 1.18 $Ca(NO_3)_2 \cdot 4H_2O$, 0.51 KNO_3, 0.14 KH_2PO_4, 0.49 $MgSO_4 \cdot 7H_2O$, and A-Z minor elements (6). Earlier reports on nitrogen nutrition of rice showed that during juvenile stages of growth, ammonium salts are superior to nitrates (3, 7, 10, 12). Therefore the adjustment to higher levels of nitrogen in the present study was made by addition of NH_4NO_3, which provided increases in both forms without imbalance in other cations (Table 1).

The seed lots were treated with Ceresan (ethyl mercuric phosphate, 5%) in closed containers 7 days before being sown. Twenty-five kernels of each variety were spaced in equal systematic sectors in the quartz sand in each crock and the seed covered with 1 in. of sand. The sand in the 4 series of crocks was maintained near saturation by watering with nutrient solutions containing the 4 levels of nitrogen (Table 1).

Three experiments of duplicated treatments were conducted, 7 rice varieties in each crock, 3 isolates of the pathogen and the control, and 4 levels of nitrogen. When the seedlings were 18–20 days old, they were inoculated with conidial suspensions in 0.5% gelatin solution applied by an atomizer at about 10 lb pres-

Table 1.—Total nitrogen and relative amounts of nitrate and ammonium nitrogen in modifications of Hoagland's nutrient solution.

Level	Total nitrogen (ppm)	NO_3 (ppm)	NH_4 (ppm)
1	105	105	0
2	161	133	28
3	217	161	56
4	329	217	112

Table 2.—Average per cent of seedlings blighted by *H. oryzae* in 7 varieties of rice grown in 0.5*N* Hoagland's solution with 4 levels of nitrogen.

Variety	Isolate of H. oryzae	Blighted seedlings (%) at nitrogen levels of 1		2		3		4	
		3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Rexoro	H2	57	70	0	0	0	0	28	50
	H3	58	67	0	0	0	0	20	50
	H5	35	55	0	0	0	0	25	51
Caloro	H2	40	60	0	0	0	0	31	72
	H3	36	54	0	0	0	0	30	70
	H5	34	55	0	0	0	0	33	67
Toro	H2	42	74	0	10	0	0	66	80
	H3	46	67	0	8	0	0	68	80
	H5	32	60	0	0	0	0	33	68
Zenith	H2	30	54	0	14	0	0	35	50
	H3	24	58	0	5	0	0	35	50
	H5	24	46	0	0	0	0	28	50
Blue Bonnet	H2	30	65	0	6	0	0	28	56
	H3	30	66	0	3	0	0	26	54
	H5	25	60	0	0	0	0	25	50
Blue Bonnet 50	H2	64	70	0	8	0	0	77	87
	H3	68	73	0	11	0	0	76	84
	H5	31	68	0	0	0	0	40	59
Av. of 6 varieties		38.1	62.3	0	3.61	0	0	39.2	62.9
B52-2579	H2	100		24	26	0	15	100	
	H3	100		18	25	0	16	100	
	H5	100		13	25	0	10	100	
Av.		100		18.3	25.3	0	13.7	100	

sure. Inoculum was prepared from cultures of the 3 isolates grown 14 days on potato-dextrose agar in Petri plates at 24°C. The 3 series of 4 crocks each with seedlings to be inoculated with each of the 3 isolates were placed in separate humidity chambers at 28°C for 12 hours before, during, and for 18 hours after inoculation. The 3 inoculated series and the controls were placed in similar separate locations in the 28°C greenhouse.

Data on disease development were taken 3 and 7 days after inoculation. They comprised: (a) percentages of blighted seedlings, (b) 10 determinations of number of spots present per 1 cm length of leaf, and (c) size of spots and lesion spread. Data were summarized and evaluated by several methods.

The effect of substances exosmosing and collecting as droplets on the lesions upon germ tube elongation of the fungus was studied by using 1-ml portions of the exudate collected from leaves of the several varieties grown at 4 levels of nitrogen. The exudate liquid was placed in sterile Van Tiegham slides inside sterile Petri dishes containing moist filter paper. A loopful of conidial suspension of culture H2 containing about 40–50 conidia was placed in the drop. The slides were incubated at 24°C for 3 hours, after which the germ tube lengths of 25 conidia chosen randomly were determined, and averages for duplicate slides calculated.

Histopathological studies on the effect of nitrogen were made of leaf sections 3 and 4 days after inoculation. Leaf sections were fixed in Nawaschin's solution, dehydrated with ethyl alcohol, embedded in paraffin, cut with a rotary microtome, and stained with Fleming's triple stain.

Experimental results.—Average data from 3 experiments on the influence of nitrogen on seedling growth, seedling mortality 3 and 7 days after inoculation, and number of leaf spots per cm of leaf 7 days after inoculation are given in Tables 2–4. The effects of different forms and levels of nitrogen on disease development are illustrated in Fig. 1–4.

Influence of nitrate and ammonium levels on disease development.—The effect of ammonium nitrogen deficiency was conspicuous in the comparison of plant and disease development in Hoagland's solution and the modified solutions. At the 105-ppm level of nitrogen supplied exclusively as nitrate, seedling growth was poor, seedlings were chlorotic, and blight and leafspot were severe. Plants showed symptoms of nitrogen deficiency from emergence on. The seedlings showed numerous small spots on leaves and leaf sheaths 24 hours after inoculation. These were initially small, 0.5–1×0.1–0.2mm, and mostly linear, but grew rapidly and within 48–72 hours coalesced to form large lesions covering more than half the leaf area. Seedlings blighted rapidly in variety B52-2579. In 3–7 days the percentage of seedling blight was high in all varieties grown without NH_4 (Table 2). The surviving seedlings were retarded in growth, and lesions were abundant on the leaves extended as well as those in the leaf whorl at the time of inoculation (Fig. 1). The seedlings in the controls developed the pale-green foliage and reduced leaf size characteristic of nitrogen deficiency.

At 161 and 217 ppm of nitrogen with ammonium salts as sources of nitrogen as well as increases in

Table 3.—Average number of spots per cm of leafblade incited by *H. oryzae* in seedlings of 7 varieties of rice grown in 0.5*N* Hoagland's solution with 4 levels of nitrogen; similar data for 3 isolates averaged.

Variety	No. leaf spots per cm of blade at nitrogen levels of			
	1	2	3	4
Rexoro	15.7	6.2	3.8	20.9
Caloro	13.8	7.1	6.2	15.5
Toro	15.0	6.0	5.8	15.3
Zenith	16.5	7.8	5.2	18.3
Blue Bonnet	18.3	8.0	5.7	14.1
Blue Bonnet 50	20.8	7.0	5.3	14.3
Av. of 6 varieties	16.7	7.0	5.4	16.4
B52-2579	Plants dead	11.5	11.1	Plants dead

nitrates, the seedlings developed vigorously and the foliage was dark green. The growth of the seedlings in the controls and in the inoculated series indicated that ammonium nitrogen was essential for good seedling growth in the saturated sand or under conditions of low aeration (3, 5, 7, 10, 12). Leaves in the inoculated series 48 hours after inoculation showed distinctly isolated, linear to ovate spots of 1–2.5×0.2–0.4 mm, and fewer than in the nitrogen-deficient or in the solution with excess NH_4 (Fig. 2–3) (Table 2). Blighted seedlings were present only in the susceptible B52-2579. Symptoms 3 and 7 days after inoculation showed some increase in seedling blight only at 161 ppm, and some enlargement of leaf spots, averaging slightly smaller at 217 ppm. With the exception of B52-2579, growth and new leaf development were similar in inoculated and control series of plants.

Seedlings at the high nitrogen level, 329 ppm, developed vigorously, with dark-green foliage. In the inoculated series, however, seedling blight and leaf spot were severe, equal to that of plants grown in NH_4 nitrogen-deficient nutrient solution (Tables 2, 3). Twenty-four hours after inoculation a large number of small brown spots of 0.5–1×0.1 mm were evident. They were too numerous to increase much in size, but leaf blight and seedling blight developed rapidly and resulted in severe damage (Fig. 4). Within 3 days, all B52-2579 seedlings were blighted and a high percentage of those in other varieties were dead. The surviving seedlings in the inoculated series were stunted.

Fig. 1-5.—**1)** Disease symptoms on seedlings of Rexoro grown at 105 ppm (NH_4-deficient) N level 4 days after inoculation with *H. oryzae*. **2–4)** Number and size of leafspots incited by *H. oryzae* grown at 3 levels of nitrogen. **2)** 161 ppm. **3)** 217 ppm. **4)** 329 ppm (high NH_4 nitrogen). **5)** Transection of leaves through lesions on leaf of Rexoro grown at 217 ppm (intermediate NH_4) N level. (×350)

The 3 isolates of *H. oryzae* incited similar disease development and disease reaction. Seedling blight was slightly less with H_5 in all the varieties excepting the susceptible B52-2579 (Table 2). Leaf spot was similar with the 3 isolates, and the data were averaged (Table 3). Data also showed that the albino mutant H_3, induced by ultraviolet light, was similar in pathogenicity to the original culture.

Rice varieties showed relatively the same trend in disease reaction at the several NH_4 levels. The susceptible B52-2579 was damaged more than the other 6 varieties at all levels. The latter varieties were not damaged greatly by disease at the 2 intermediate levels, and they showed some evidence, though not significant, of a differential reaction at both the high and low NH_4 levels.

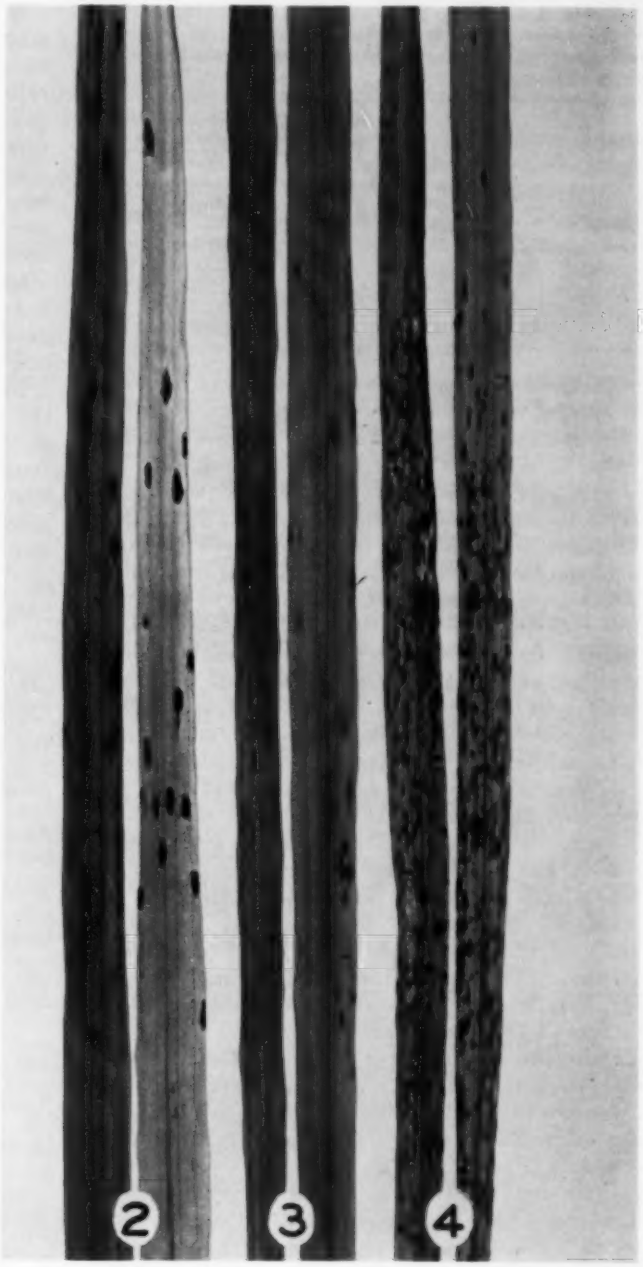
Influence of exudate solutions on germ tube elongation of H. oryzae.—The data indicated that the level of NH_4 influenced disease development. The possible influence of change in host cell sap at the infection sites on mycelial growth of the fungus was investigated by determining the influence of the exudate accumulating at these sites under high humidity on germ tube elongation (Table 5).

Average germ tube elongation was highest in the exudate from the plants grown in the high NH_4 level and decreased with decreases in the nitrogen in the 2 intermediate series. It was high again in the NH_4 nitrogen-deficient nutrient solution. Germ tube elongation in the exudates from the infection sites appeared to be correlated with number of lesions, leaf tissue damage, and number of blighted seedlings. Since both cell sap composition and cell permeability were

Table 4.—Average seedling height at inoculation and number of leaves per seedling of 7 varieties of rice grown in 0.5*N* Hoagland's solution with 4 levels of nitrogen.

Variety	Nitrogen levels							
	1		2		3		4	
	Plant height (cm)	Leaves (no.)	Plant height (cm)	Leaves (no.)	Plant height (cm)	Leaves (no.)	Plant height (cm)	Leaves (no.)
Rexoro	10	3	25	3	30	4	35	4
Caloro	10	3	24	3	30	4	34	4
Toro	10	3	17	3	25	3–4	27	4
Zenith	11	3	20	3	28	4	33	4
Blue Bonnet	7	3	12.5	3	15	3	17.5	3–4
Blue Bonnet 50	8	3	13	3	15	3	17	3–4
B 52-2579	11	3	17	3	22	3–4	32	4
Av.	9.57	3	18.35	3	23.57	3.6	27.93	4.2

1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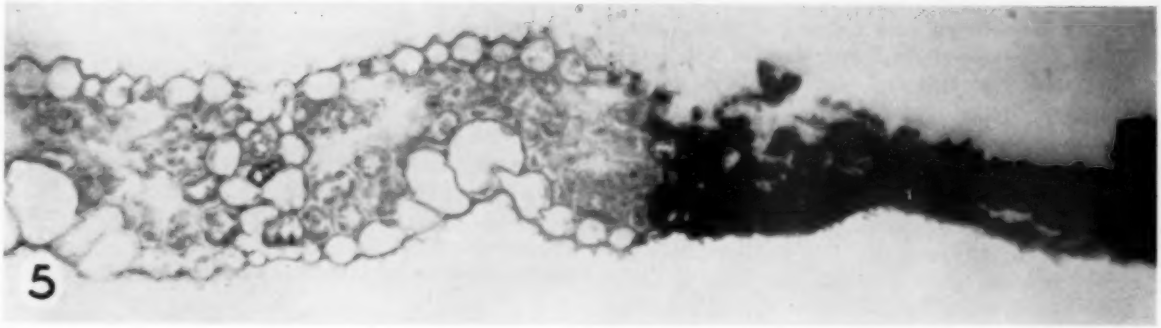
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Table 5.—Average germ tube length (μ) from conidia of *H. oryzae* grown in exudate collected from lesions on rice seedlings grown in 0.5*N* Hoagland's solution with 4 levels of nitrogen

Variety	Germ tube length in lesion exudates from plants at nitrogen levels of:			
	1	2	3	4
Rexoro	26.3	13.5	20.6	32.9
Caloro	26.6	13.9	20.7	33.2
Toro	28.0	12.2	20.4	33.1
Zenith	32.7	17.8	20.8	35.3
Blue Bonnet	30.3	20.1	22.6	32.0
Blue Bonnet 50	26.4	18.0	20.2	27.4
Av.	28.38	16.25	20.9	32.3

probably involved in these reactions, data cannot be interpreted without further investigations.

Histopathological reaction to nitrogen levels.—Leaves of seedlings grown in the solution with NO_3 showed poor development of mesophyll and bundle sheath in contrast with those grown with NH_4. Seedling leaves from the high NH_4 level developed larger epidermal motor cells and mesophyll cells with thinner walls than in the others. Cross sections of the leaves from both the high and deficient NH_4 levels showed that the damage was not confined exclusively to mesophyll cells, but that vascular elements and bundle sheath were affected also. The cells of the diseased tissues showed collapse, some shrinkage, and an affinity for safranin. Similar necrosis and staining reaction were observed by Green and Dickson (4) with *Septoria* leaf blotch of barley. In leaves of seedlings grown at the intermediate NH_4 nitrogen levels, fungal invasion and necrosis were restricted largely to mesophyll cells (Fig. 5). Tulles (11) observed that spread of the fungus was impeded by bundle sheath in resistant varieties, but not in susceptible varieties. The present investigations indicated that high NH_4 level caused a similar response in all but the very susceptible variety. The deposit of gum-like substances in cells adjacent to invaded tissue reported in resistant varieties (11) was not observed.

Discussion.—Both seedling development and disease reaction indicated that NO_3 nitrogen in Hoagland's solution was not utilized by rice. The seedlings were very stunted and chlorotic, and blighted severely when grown in 0.5*N* Hoagland's solution. The addition of 3 levels of NH_4NO_3 to the solution in which 28, 56, and 112 ppm NH_4 were supplied corrected the nitrogen deficiency and reduced disease damage incited by *H. oryzae* at the 2 intermediate NH_4 levels. However, both leaf spot and seedling blight were increased greatly at the high NH_4 level, based on the reaction of 6 varieties of rice. The superiority of NH_4 nitrogen to NO_3, especially under low aeration, is reported (3, 7, 10, 12), although data on the influence of low and high levels of available N on disease development in rice is limited.

Increased susceptibility was indicated by the altered anatomical structures of the tissues and by rapid necrosis at both NH_4-deficient and high levels. Increased exosmosis of nutrients into the exudate at the infection site accelerated germ tube growth at both N levels. These relations suggest that increased pathogenicity was associated with soluble materials in the cell sap.

In recent years in various rice-growing countries of the world, attempts have been made to increase the yield of rice by liberal application of nitrogenous fertilizers both in the nursery and later growth stages. The present investigation points out the undesirable consequences of injudicious application of nitrogenous fertilizers, particularly in areas where Helminthosporium blight is prevalent.—Department of Plant Pathology, University of Wisconsin, Madison.

LITERATURE CITED

1. Anonymous. Agricultural Research. 1954. Rept. India Council Agr. Research 1952-53:2-45.
2. Dickson, J. G. 1956. Diseases of field crops. McGraw-Hill Book Company, Inc., New York. 517 p.
3. Espino, R. B. 1920. Some aspects of the salt requirements of young rice plants. Philippine J. Sci. 16:455-525.
4. Green, G. J., and J. G. Dickson. 1957. Pathological histology and varietal reactions in Septoria leaf blotch of barley. Phytopathology 47:73-78.
5. Hashioka, Y., and M. Makino. 1956. Relation of nitrogen nutrition of rice plants to the susceptibility of four foliage diseases. Research Bull. Fac. Agr. Gifu Univ. 6:58-66.
6. Hoagland, D. R., and D. I. Arnon. 1938. The water culture method of growing plants without soil. Calif. Univ. Agr. Expt. Sta. Circ. 347-36-39.
7. Kelley, W. P. 1911. The assimilation of nitrogen by rice. Hawaii Agr. Expt. Sta. Bull. 24 p.
8. Mallamaire, A. 1949. Maladies plantes parasites et plantes infestantes des riz cultives en Afrique Occidentale. Agron. trop. Nogent-sur-Marne 4:77-80.
9. Muller, A. S. 1950. Report on a seedling rice disease at Tiquisate, Guatemala, C. A. Plant Disease Reptr. 34:156.
10. Trelease, S. F., and P. Paulino. 1920. The effect on the growth of rice of the addition of ammonium and nitrate salts to soil cultures. Philippine J. Agr. 8:293-313.
11. Tullis, E. C. 1935. Histological studies of rice leaves infected with Helminthosporium oryzae. J. Agr. Research 50:81-90.
12. Willis, L., and J. O. Carrero. 1923. Influence of some nitrogenous fertilizers on the development of chlorosis in rice. J. Agr. Research 24:621-640.

INDUCED MUTATION AND CYTOLOGICAL INVESTIGATIONS IN HELMINTHOSPORIUM ORYZAE

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SUMMARY

Ultraviolet irradiation of germinating conidia and young hyphal fragments of a dark olivaceous conidial isolate of *Helminthosporium oryzae* induced albino mutants without apparent changes in conidial production and pathogenicity. Fine and coarse mycelial types were also recovered. A strain of the fungus was found in which mutation for pigment loss was not induced by the treatments used. The albino mutants were used to study the heterokaryotic condition of the cells of the mycelium and reassortment of nuclei through anastomosis of cells arising from germtubes from black and albino conidia. Cytological studies showed that the apical and intermediate cells of conidia are multinucleate. Germination was bipolar, and nuclei were numerous in the germtube and in the growing apex and young hyphal cells. Older cells of the mycelium were occasionally uninucleate. Hyphal cell anastomoses and nuclear exchange were observed in germtubes and later stages of growth. At septation, conidiophore initials contained one or more nuclei. Apical cells of the conidiophore and the conidial initials were multinucleate.

Helminthosporium oryzae Breda de Haan,[1] like other species of the genus, shows variability in nature and in culture. Ocfemia (11) and Nisikado (10) first reported variation in the fungus from different areas. Tochinai and Sakamoto (18) noted specialization in pathogenicity and differences in physiology and morphology. Matswura (9) reported that monosporous cultures of *H. oryzae* produced white sectors that remained stable at 28°C but reverted at higher or lower temperatures. These saltant strains formed no conidia and showed differences in pathogenicity. Frequency of saltation was reduced on exposure to ultraviolet light or X rays.

The nuclear condition and the mechanism of variation in this fungus have apparently not been investigated. The albino mutant forms, in which, apparently, only the locus for pigment formation was modified, provided material for cytological study of the fungus.

Materials and methods.—The present investigations used 2 isolates of the species: H_1, with vigorous, aerial mycelial growth of light-gray color and sparse conidial production (Fig. 9), and H_2, with black mycelial growth and profuse sporulation (Fig. 1). Germinating conidia and young hyphae suspended in water in sterile Petri plates were irradiated under a Westinghouse mercury vapor Sterilamp for 12–18 minutes. Aliquots of the suspensions were transferred, spread over the surface of malt-yeast extract agar in Petri plates, and incubated at 24°C. Each colony showing variation in pigmentation or colony type was transferred to a Petri plate of the malt-yeast extract agar. The clones were observed during several days' growth, and mass transfers were made from the contrasting types or from the sectors of clones. The relative stability of the clones was evaluated through several transfers to both malt-yeast and potato-cerelose agars.

The cytology of the mycelium and asexual reproductive phase of the fungus was studied with the cellophane square method of Roane (13) and Knox-Davies (8). The material was fixed in Carnoy's fluid and stained with Giemsa following hydrolysis by 1*N* HCl and mounted in *M*/5 KH_2PO_4 solution adjusted to pH 6.9, as recommended by Hrushovetz (7) and Knox-Davies (8).

Mutation studies.—Three albino mutants of H_2 were obtained by exposing germinating conidia and young hyphal fragments to ultraviolet radiation. The initial albino culture grew well, with floccose mycelial growth and no sectoring or pigment formation, at 8, 24, and 28°C (Fig. 2). Mycelium consisted of coarse, thick-walled (Fig. 3) and fine thin-walled hyphae (Fig. 4). Typical conidia as well as a few 1–3-celled conidia formed readily in a number of media. During the isolation of subcultures, a second albino type was obtained with sectors or patches of black, white, or intermediate colors (Fig. 5). Later, a black appressed mycelial growth with dark-gray conidia produced a floccose albino sector with light-gray to albino conidia (Fig. 6). On germination, conidia produced colonies similar in type of growth to original cultures. Irradiation of the germinating conidia and young hyphal fragments of the light-gray to albino resulted in a uniformly gray (Fig. 7) and black to albino types (Fig. 8).

Isolates of the fungus were obtained in which no mutation was induced by exposures to ultraviolet radiation for as long as 21 minutes. These all showed the type of black mycelial growth with profuse sporulation similar to that of isolate H_2.

Irradiation of young hyphal fragments of isolate H_1 induced the formation of a light-gray to buff-colored mycelial type (Fig. 10) from which the different mycelial types characteristic of H_2 were recovered by hyphal transfers.

Exposure to ultraviolet irradiation for less than

[1] *Bipolaris oryzae* (B. de Haan) Shoemaker, Can. J. Botany 37: 879-887, 1959.

12 minutes produced no evidence of mutant types. Similarly, ungerminated conidia and fragments of old hyphae did not respond to irradiation.

Stable albino and dark types were grown in association on a number of media and analyzed for intermediate types resulting from hyphal anastomoses. Conidiophores with conidia of varying shades of gray were formed on several areas of the mycelium (Fig. 11). Mycelial isolates from these conidia were dark, light-gray, and albino (Fig. 12).

Cytological studies.—Apical and intermediate cells of the albino conidia were multinucleate (Fig. 14). Germination of the conidia was bipolar (Fig. 13). During the early phase of germination, migration of nuclei into the germtube was studied and later nuclear divisions were observed (Fig. 13, 14). Apical cells and cells of the young growing hyphae were multinucleate (Fig. 16). The number of nuclei varied from 1 to many in cells of mature mycelium (Fig. 18). Hyphal fusions were evident in older hyphae that grew parallel on the cellophane plates (Fig. 15). Generally conidiophores at initial septum formation contained a number of nuclei (Fig. 19, 20). Occasionally, uninucleate conidiophore initials were observed (Fig. 17). Septum formation in the development of conodiophore initials was delayed in a few cases (Fig. 20). Apical cells of the conidiophore and the conidial initials were generally multinucleate.

Discussion.—Albino mutants of *H. oryzae* that resembled the parental isolate in other essential features were obtained by ultraviolet irradiation. The relative frequency of such mutations without apparent changes in other characters is indicative of the mutability of this locus as well as its apparent independence from loci conditioning pathogenicity, conidial production, etc. The study of chemical mutants in these clones is essential for an understanding of the genetic mechanisms involved.

Variability in *Helminthosporium* spp. has been attributed to the heterokaryotic condition of the asexual mycelium and mutation (2, 3, 4, 5, 14, 16, 17). Other phenomena also function in determining variability, as demonstrated by the several investigations of comparative differences in the characteristics of cultures from hyphal tips originating from the same fungus clone (14, 16). The relatively recent concept of the mechanism conditioning variability in microorganisms (1, 12, 15) briefly summarized as heteroalleles, cistron, etc., in which, on the gene basis, intragenic crossing over is demonstrable at a locus, appears applicable in explaining the variabiilty demonstrated in these heterokaryotic fungi.

These preliminary cytological investigations of this species present evidence similar to that obtained by others on perpetuation of the heterokaryon through conidial production (8, 14). The data indicate both mono- and multinuclear transfer into the asexual conidia. In every study reported, concentration of nuclei

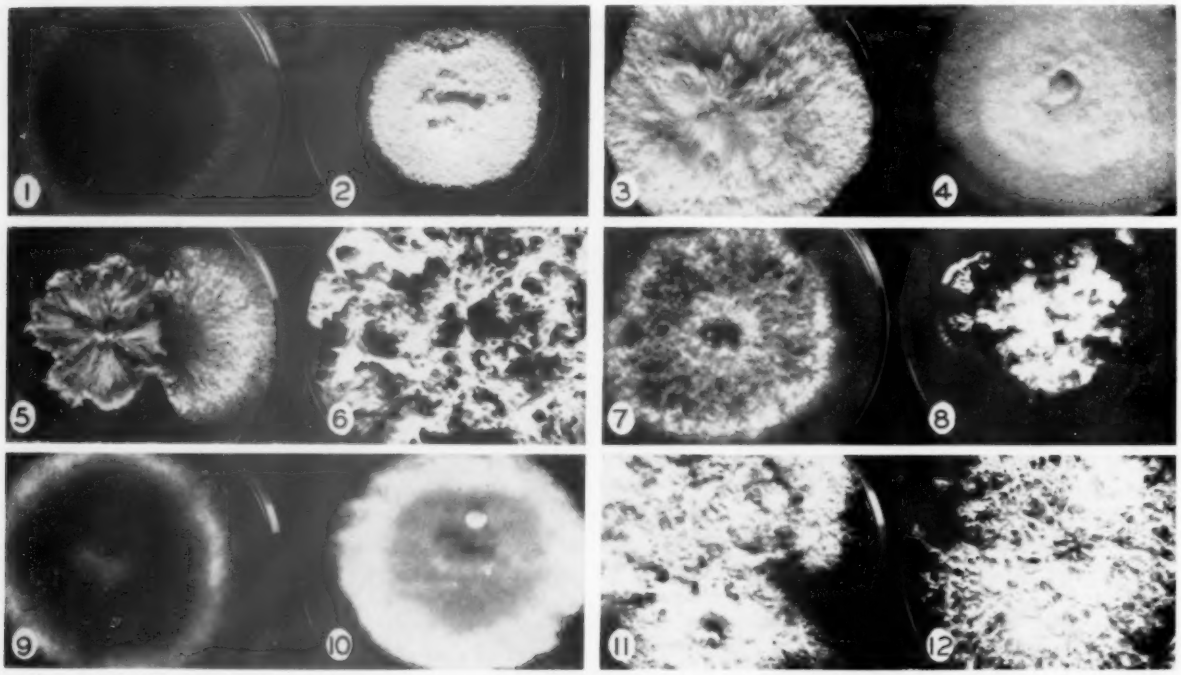

Fig. 1–12.—Representative cultures on malt extract agar at 24°C of the different types recovered from induced mutation by ultraviolet radiation in isolates H_1 and H_2. **1**) 4-day-old culture of H_2. **2**) 4-day-old culture of albino mutant of H_2. **3**) 5-day-old culture of H_2 albino, coarse mycelial type. **4**) 5-day-old culture of H_2 albino, fine mycelial type. **5**) 5-day-old culture of albino I showing sectors. **6**) 14-day-old culture of albino I showing black and albino mycelia. **7–8**) 5-day-old culture of a gray type derived from albino I after ultraviolet radiation. **9**) 5-day-old culture of isolate H_1. **10**) 5-day-old, light colored mutant of H_1. **11**) Culture of stable black and albino on potato cerelose agar showing formation of conidiophores at the junction of two cultural types after 2 weeks' growth. **12**) Black parental and albino types recovered after growing black and albino together on artificial media.

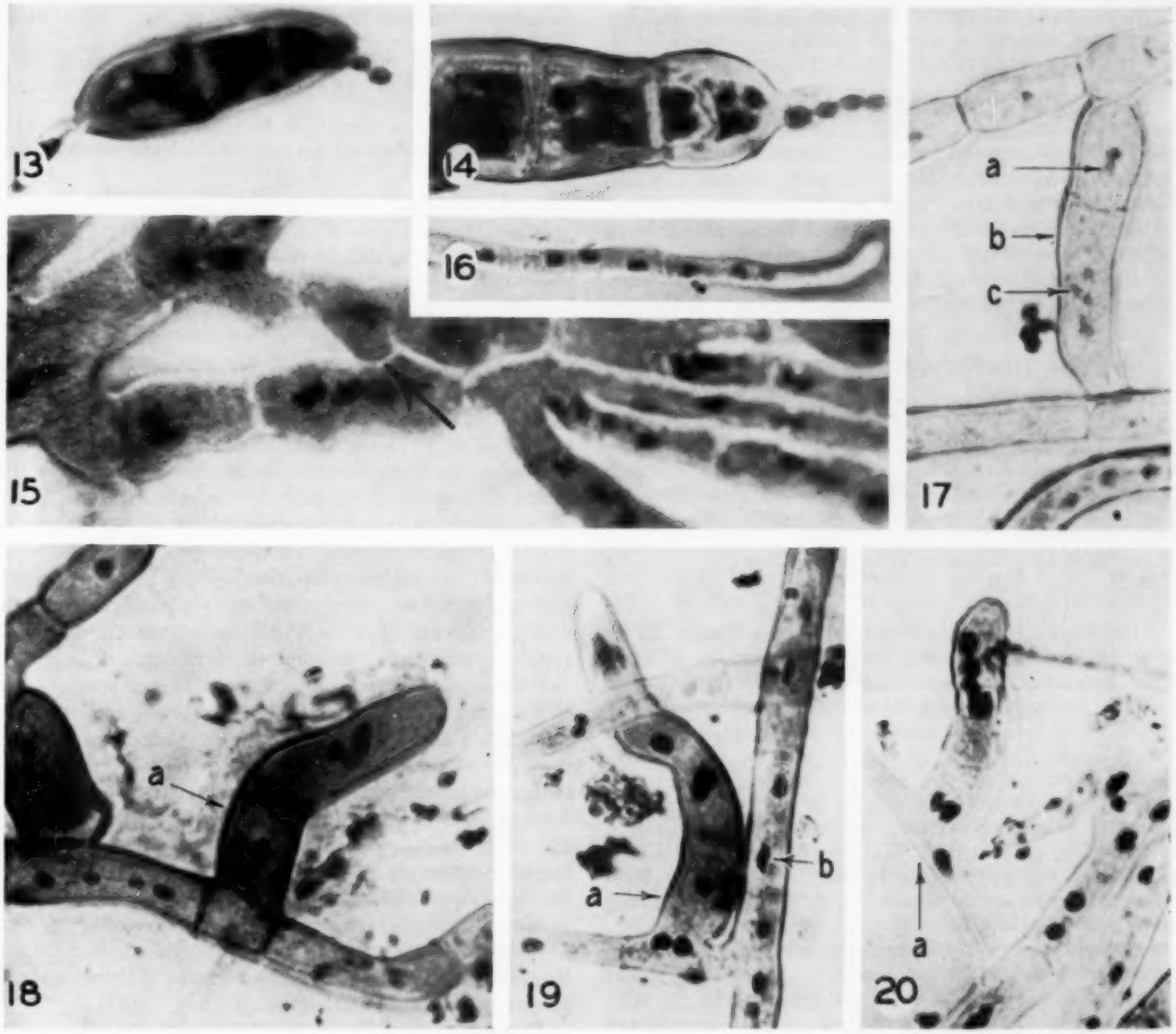

Fig. 13–20.—Showing nuclear condition in conidia, conidiophore and mycelium of albino mutant of H_8. (×1600). **13)** Migration of nuclei into germtube at the initiation of germination. **14)** Multinucleate, intermediate, and apical cells of conidia and migration of nuclei into the germtube. **15)** Fusion of hyphal cells growing parallel on cellophane strips. **16)** Multinucleate condition of young hyphal and apical cells. **17)** Uninucleate hyphal cell and conidiophore with a single nucleus originating from uninucleate hyphal cell (b). (a–c) dividing nuclei in the conidiophore. **18)** Hyphal cells of old mycelium with 1 to many nuclei. (a) development of multinucleate conidiophore initial. **19)** Multinucleate apical and basal cell of the conidiophore (a). (b) elongated nucleus in the thin-walled hyphae. **20)** Delay in the formation of septum during development of conidiophore (a).

or nuclearlike aggregates in the apices of hyphae and conidiophores makes definition difficult. Several processes are apparently involved in conditioning variability in this species causing seedling, leaf, and kernel blight of rice.—Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

LITERATURE CITED

1. Benzer, S. 1957. The elementary units of heredity. Symposium on the chemical basis of heredity. p. 70-93. Johns Hopkins Press.

2. Christensen, J. J., and F. R. Davies. 1937. Nature of variation in Helminthosporium sativum. Mycologia 29:85-99.

3. Dickinson, S. 1932. The nature of saltation in Fusarium and Helminthosporium. Minn. Univ. Agr. Expt. Sta. Tech. Bull. 88, 42 p.

4. Graham, T. W. 1935. Nuclear phenomena in Helminthosporium gramineum. Phytopathology 25:284-286.

5. Greaney, F. J., and J. E. Machacek. 1933. The production of a white fertile saltant of Helminthosporium sativum by means of ultraviolet radiation. Phytopathology 23:379-383.

6. Hiroe, I. 1937. Experimental studies on the saltation in fungi parasitic on plants. Mem. Tottori Agr. Coll. 5:272.

7. Hrushovetz, S. B. 1956. Cytological studies of Helminthosporium sativum. Can. J. Botany 34:321-327.

8. Knox-Davies, P. S. 1959. The cytology and genetics of Helminthosporium turcicum and its ascigerous stage Trichometasphaeria turcica. Ph.D. thesis, Univ. of Wisconsin. 117 p.

9. Matswura, I. 1930. Experimental studies on the saltation in fungi (preliminary report). IV. On the saltation of Ophiobolus miyabeanus Ito and Kuribayashi parasitic on rice plant. III Agr. and Hort. 5:1477-1496. (In Japanese)

10. Nisikado, Y. 1927. Comparative studies on Helminthosporium diseases of rice in the Pacific regions. Ber. Ohara Inst. landwirtsch. Forsch. Kuraschiki Japan 3:425-440.

11. Ocfemia, G. O. 1924. The Helminthosporium disease of rice occurring in the Southern United States and in the Philippines. Am. J. Botany 11:385-408.

12. Pontecorvo, G. 1958. Trends in genetic analysis. Columbia Univ. Press., New York. 145 p.

13. Roane, C. W. 1952. A method for preparing fungi for cytological studies. (Abstr.) Phytopathology 42:480.

14. Robert, Alice L. 1952. Cultural and pathogenic variability in single-conidial and hyphal-tip isolates of Helminthosporium turcicum Pass. U. S. Dept. Agr. Tech. Bull. 1058. 53 p.

15. Roman, H. 1956. Studies of gene mutation in Saccharomyces. Cold Spring Harbor Symposia on Quantitative Biology 21:175-186.

16. Shands, H. L., and J. G. Dickson. 1934. Variation in the hyphal tip cultures from conidia of Helminthosporium gramineum. Phytopathology 24:559-560.

17. Tinline, R. D. 1954. Physiology, pathogenicity and genetics of Helminthosporium sativum. Ph.D. thesis, Univ. of Wisconsin. 105 p.

18. Tochinai, Y., and M. Sakamoto. 1937. Studies on the physiologic specialization in Ophiobolus miyabeanus Ito et Kuribayashi. J. Fac. Agr. Hokkaido. Imp. Univ. 51:1-96.

THE HISTOPATHOLOGY OF ROOT-KNOT NEMATODE INFECTIONS IN SOYBEANS

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SUMMARY

Histological response to infection was studied in 19 soybean varieties infected with 2 populations of root-knot nematodes. Sequence of giant cell development in very young roots of a favorable host variety (Richland) is as follows: intense cell multiplication about larval head, followed by hypertrophy of cells immediately surrounding the mouth; cell wall dissolution proceeding outward from hypertrophied cells, resulting in multinucleate units, the giant cells. Nuclear division within giant cells is rare. Mature giant cells are classified into 4 types according to morphology: Type 1 refers to a hypersensitive reaction in which cells immediately around the larva die, and no further development occurs. Type 2 cells undergo moderate cell fusion and display unusually great numbers of cell inclusions of peculiar kinds. Type 3 cells are very large, with many nuclei, and with a diffuse, highly vacuolated cytoplasm. Types 1, 2, and 3 are always associated with poorly developed parasites with few eggs. Type 4 giant cells consist of large, thick-walled multinucleate units with dense cytoplasm and few cell inclusions. They are associated with rapid parasite growth and abundant egg production. A clue to one aspect of resistance is that variety S-100, an unsuitable host, lacks the initial abundant mitoses that accompany giant cell production in a suitable host. Giant cells of type 4 do not develop.

Introduction.—The formation of characteristic multinucleate structures by plants infected with root-knot nematodes is well recorded in the literature (2, 7). These giant cells are believed to arise in response to substances emanating from the parasite's head and to provide nutriment to the parasite. In spite of the striking appearance of giant cells and their obvious importance in host reaction to infection there are no detailed studies correlating their character with growth of the parasite. The present paper surveys giant cell morphology and associated phenomena in 19 varieties of soybeans (*Glycine max* (L.) Merr.) exposed to *Meloidogyne incognita incognita* (Kofoid & White, 1919) Chitwood, 1949, and *M. incognita acrita* Chitwood, 1949. A report of galling and parasite reproduction in this material has been presented (3) and the work has been summarized (4). In the present paper, distinct morphological types of giant cells are described; one type is consistently correlated with rapid growth and reproduction of the parasite.

Materials and methods.—Infections were produced in the greenhouse by adding to sterilized soil in 4-in. pots ample amounts of inoculum consisting of chopped roots infected with root-knot nematodes. Seeds of the soybean varieties were planted at the time of inoculation and allowed to grow until a susceptible variety (Richland) showed well-developed egg masses. Then samples of roots of all the varieties were fixed in Randolph's modification of Navashin's fluid and embedded in paraffin after dehydration in tertiary butyl alcohol (6). Cross sections were cut at 10 μ and stained with safranin and fast-green. Histochemical procedures followed standard methods (10).

A series of timed infections was prepared with seedlings of Richland and S-100 soybeans infected with *M. arenaria arenaria* (Neal, 1889) Chitwood, 1949. Roots were exposed to infection for 24 hours, then washed and grown in plastic bags filled with a 1:1 mixture of hand-rubbed peat and fine beach sand (1). The bags were pressed flat, then bound between sheets of corrugated cardboard and kept upright in a simple growth chamber at 24–27°C under constant fluorescent illumination.

Anatomy of the healthy soybean root.—The soybean root system consists of a taproot and its branches. The taproot, or primary root, produces secondary roots, which in turn give rise to further branch roots. The taproot and its prominent branches are typically tetrarch (Fig. 1). When root tissues are in the primary state the pericycle is typically uniseriate except at the protoxylem poles, where it may be multiseriate. The first indication of the development of secondary tissues is the appearance of vascular cambial strands between the protoxylem poles of the root. Lateral extension of the vascular cambial strands continues until the complete cambial ring is formed. The pattern of

development for secondary phloem and xylem tissues in the soybean root is typical of the roots of dicotyledons. Secondary phloem develops centrifugally, and secondary xylem centripetally, from the vascular cambium. Just preceding or during the development of secondary tissues, clumps of fibers develop in the phloem in the region of the protophloem poles. These fibers are inside the crushed protophloem and can be classed as phloem fibers (5). In well-developed secondary roots the pericycle is often multiseriate, sometimes reaching a thickness of 7 layers. Roots with extensive development of secondary tissues may also have a well-developed pith in the stele. The size of the pith is variable. The pith in some cases is large enough to fill most of the center of the stele, and in other cases may consist of only a few cells. The smaller branch, or lateral, roots are made up entirely of primary tissues, and usually do not produce secondary tissues from a vascular cambium. Primary roots may be diarch, triarch, or tetrarch, but are typically tetrarch.

Early development of giant cells.—In Richland variety of soybean, infective larvae make their way through the cortex to the vicinity of the protoxylem poles. Roots fixed within 24 hours of exposure to larvae show no evidence of cortex cell damage. Larvae are present both intercellularly and intracellularly. Soon after arriving at their destination, larvae induce 3 types of plant response that lead to giant cell formation: 1) There is a region of intense cell multiplication about the head of the larva in the 3-day and subsequent series. Cambium fails to differentiate in a sector of developing root, so that the mature infected root often exhibits a wedge of parenchyma tissue extending acropetally and basipetally beyond the giant cells. 2) Along with the stimulation of mitoses, enlargement of a few cells about the larval head marks the beginning of giant cell formation. The hypertrophied cells are in direct contact with the nematode's head and probably represent cells into which secretions have been injected. In sections taken 3 or 5 days after exposure to infection, the nuclei, especially the nucleoli, appear deeply stained. Fig. 2 shows one area of hypertrophy and hyperplasia close to the head of the larva 3 days after exposure to infection. 3) The sections taken 7 and 9 days after exposure to larvae offer clear evidence of the incorporation of parenchyma cells into the growing giant cells. Cell walls of groups of cells adjacent to a young giant cell appear to be dissolved. The walls of giant cells are thicker than those of neighboring cells except in regions of cell incorporation.

Further development proceeds by dissolution of cell walls and incorporation of the contents of neighboring parenchyma cells into growing giant cells. The occurrence of mitoses within giant cells is limited to the first few days of development, and probably represents part of the mitotic flare induced by the nematode, so that there is no specific mitotic activity within giant cells. Mitoses continue in cells at either end of the group of giant cells. The abnormal parenchyma cells may extend for more than 300 μ in both directions along the root axis from the nematode's head. When many larvae invade the same root, normal development may be severely disturbed so that only remnants of xylem and phloem tissue remain.

Giant cells have been seen in young sections in both protoxylem and protophloem parenchyma. In mature sections they appear in parenchyma tissue within the secondary xylem, within the secondary phloem, and in the enlarged pericycle. In our material no giant cells were found in cortex. This contrasts with root-knot in tomato and with *Heterodera* infections, where giant cells do occur in cortex as well as in stele.

Thickened walls of cells surrounding the larval head are first evident in the sections taken 3 days after exposure to larvae. In older roots, particularly at the ends of the group of stimulated cells, there are areas that show thickened walls on adjacent sides of several cells. These areas suggest a response to a stimulating substance seeping between cells that induces cell wall thickening. Pronounced thickening of the walls of growing giant cells first appears in our series on the 9th day after exposure to larvae. The heavy wall of mature giant cells consists of a layer of granular, green-staining material that tests positively for cellulose and pectin, but negatively for lignin, suberin, and starch, and does not stain with ninhydrin.

Mature giant cells.—Throughout the series of soybean varieties ranging from those in which nematodes were reproducing actively to those in which nematodes made very little growth, correlation was striking between the morphology of giant cells and the growth rate of the nematodes. Four giant cell types are evident. Poorly developed nematodes occur in association with giant cell types 1, 2, and 3, but not with type 4, which is always associated with fully mature females and large egg masses.

Type 1.—The plant responds with marked necrosis of cells immediately around the head of the larva (Fig. 3). Very little cell enlargement and no incorporation of cells into a giant cell are evident. This corresponds to the hypersensitivity exhibited by many plants invaded by fungi.

Type 2.—Some enlargement of giant cells occurs through the incorporation of adjacent cells, but the giant cell never attains the size of types 3 and 4. The conspicuous feature of type 2 giant cells is the presence of abundant cell inclusions of various forms (Fig. 4, 5). The cell may contain a profusion of spirals, strands, or lobed matter, all of which stain green with fast-green and give positive tests for cellulose and pectin. These cells apparently produce more than the normal amount of wall material, which then fails to be deposited on walls but remains visible throughout the cytoplasm. Type 2 cells are often seen with some red-staining material as well, which we have interpreted as evidence of necrosis. Some type 2 cells occur in all varieties, and they have been noted in the timed series as well as in mature roots.

Type 3.—These are large giant cells almost equal in size to the optimal type for nematode growth but

they differ in two characters from type 4: a) the walls do not show the dense aggregations of green-staining granular material characteristic of type 4; b) the cytoplasm is highly vacuolated and appears generally less dense than in the optimal type (Fig. 6). Nuclear clumps as described in type 4 may be present.

Type 4.—The mature giant cell complex of soybeans usually consists of a group of 5–9 multinucleate cells. The cytoplasm is evenly granular, gray, and dense in stained sections. The wall consists of 2 conspicuous parts. The outer layer is clear, stains green, and represents that part of the wall first thickened in early giant cells. It is birefringent. In some sections, areas resembling pit fields are present in this layer. The inner layer is variable in thickness, does not exhibit birefringence, and is frequently more or less beaded in appearance, with adjacent cells showing symmetrical aggregations of green-staining material (Fig. 7). After digestion of cellulose by Schweitzer's reagent, the inner wall shows a profusion of small granules.

As many as 150 nuclei have been found in fully developed giant cells. One type is very large, measuring up to 21×16 μ in cross section, and is oval-shaped with a large brilliantly red-staining nucleolus and coarse clumps of red matter. The nucleoli are as large as whole nuclei of adjacent parenchyma cells. The large nuclei are scattered throughout the cytoplasm, but tend to be aggregated somewhat toward the center of the giant cell. The second type of nucleus is much smaller and is very often aggregated in a single cluster of pyriform nuclei radiating out in all directions from a center (Fig. 8). Such clusters are located toward the center of a giant cell and may contain as many as 75 nuclei, but usually contain fewer. Some giant cells do not exhibit this phenomenon, which has been seen commonly in many soybean varieties. The origin of the two classes of nuclei is obscure. Large oval nuclei may originate from the initial hypertrophied cells, which show very enlarged nuclei in the 3-day sections. The smaller nuclei probably originate in actively growing cells at the borders of enlarging giant cells. These actively dividing cells are a prominent feature of the series of timed infections. In roots fixed 53 days after the seed was planted, giant cells were often associated with groups of long narrow cells. The nuclei in these are of the same general appearance as the small nuclei in the giant cell. No explanation is offered for the clusters of small nuclei.

Long narrow rods measuring up to 70 μ long and 1 μ wide are a prominent feature of some giant cells (Fig. 9). In the Adams soybean, some giant cells contained about 25 such rods. The rods appear to have some internal structure, but they are so narrow that little detail can be distinguished. Němec (9) called them mitochondria, but they do not appear to be mitochondria, both by morphology and because they fail to break down in fresh tissue after being heated to 55°C. Rods do not appear in all giant cells of a particular host, nor do they appear in all hosts, probably because fixation was not specifically designed for preservation of these structures. It is possible that they represent coagulated secretions from the nematode (8). They occur most often in the immediate vicinity of the nuclear clumps.

Some empty giant cells have been observed, particularly in Capital soybeans (Fig. 10). These have somewhat thickened walls and contain a few remnants of scattered nuclei. They probably represent dead giant cells.

Two examples were noted of a structure not corresponding to a stage in the formation of giant cells. These were islands of hypertrophied thin-walled cells surrounded by what appear to be giant cell walls (Fig. 11). It is difficult to account for such structures.

Giant cells of various types are often present in the same section. In soybean variety Dorman, for example, a cluster of giant cells associated with one nematode typically has one large giant cell of type 4 and several giant cells of type 2, but no necrosis. In Richland, a very favorable host, types 2, 3, and 4 are associated with female nematodes. In general, the types of giant cells, and especially the frequency of occurrence of each type in a set of serial sections from one piece of root of a given variety, is correlated with the rating of this host-parasite combination given in the first paper (3). Exceptions are variety Dorman infected with *M. incognita incognita*, which is apparently a more favorable combination than previously reported, since many egg masses occur internally, and variety Roanoke infected with *M. incognita incognita*, for which an error was recorded. The original preserved roots of this combination have moderately abundant, small egg masses rather than no eggs, as reported.

The question naturally arises whether the sections examined are typical of the variety. A set of 5 free-hand sections was therefore made from the original roots preserved in 5% formalin. Each section was taken from a separate gall and, whenever possible, from a separate plant, to give a random sample of giant cell types. Results closely parallel the results of

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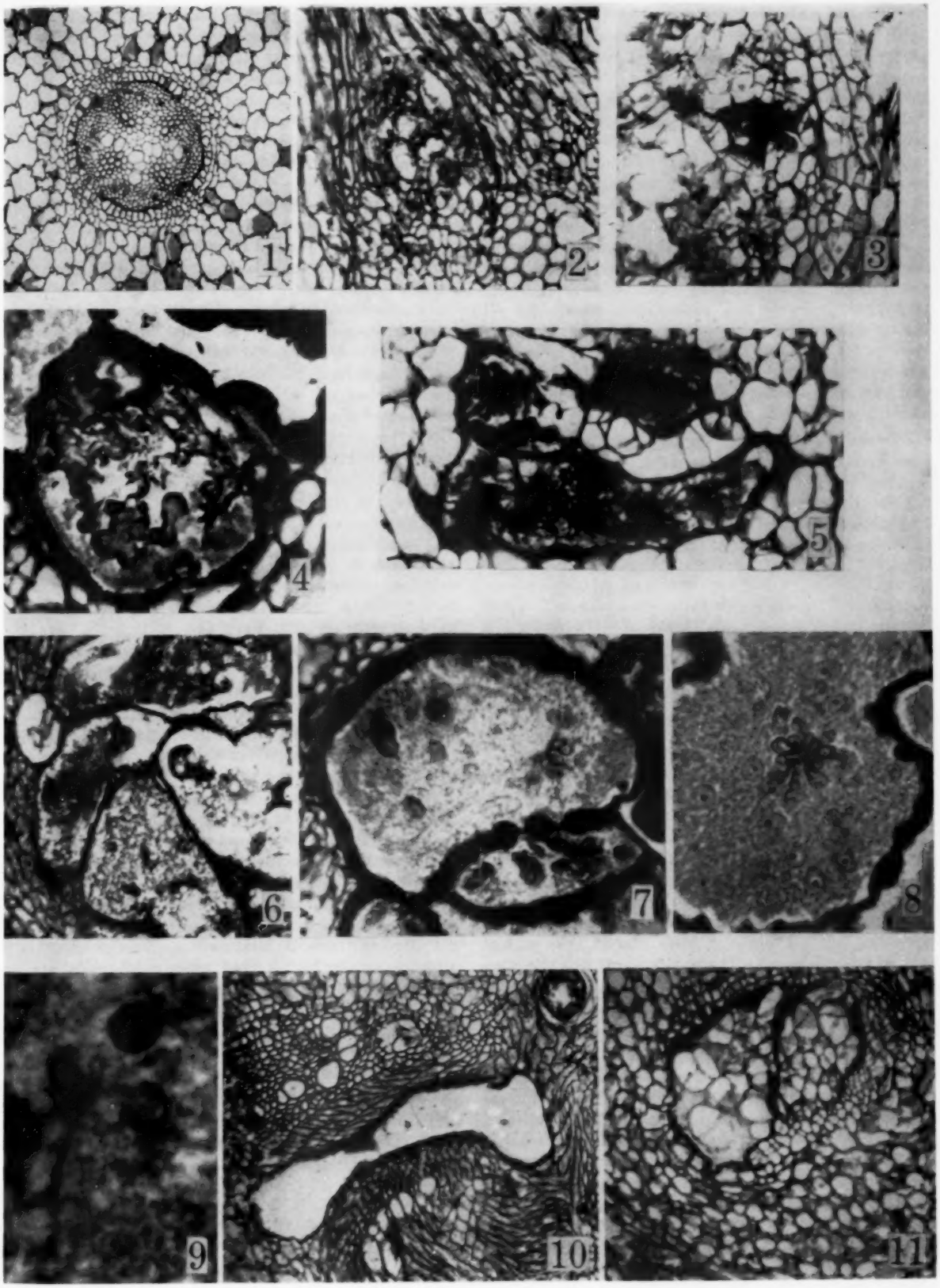
Fig. 1–11.—**1)** Primary root of uninfected soybean variety S-100 (×73). **2)** Richland soybean root fixed 3 days after exposure to *M. arenaria arenaria* larvae, showing initial hypertrophy and hyperplasia (×191). **3)** Type 1 response. Head of larva surrounded by dead cells in Blackhawk soybean infected with *M. incognita incognita* (×160). **4)** Type 2 giant cells, showing lobed cell inclusions in Roanoke soybean infected with *M. incognita acrita* (×535). **5)** Type 2 giant cell showing spiral shaped cell inclusions in Blackhawk soybean infected with *M. incognita incognita* (×405). **6)** Type 3 giant cells showing vacuolated cytoplasm and relatively thin walls in Adams soybean infected with *M. incognita acrita* (×185). **7)** Type 4 giant cell in Richland soybean infected with *M. incognita incognita* (×291). **8)** Nuclear cluster from type 4 giant cell in Adams soybean infected with *M. incognita acrita* (×641). **9)** Cell inclusion in Type 4 giant cell in Adams soybean infected with *M. incognita incognita* (×1236). **10)** Empty giant cell in Capital soybean infected with *M. incognita acrita* (×104). **11)** Islands of thin-walled parenchyma cells associated with nematode infections in Lincoln soybean infected with *M. incognita incognita* (×92).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the material embedded in paraffin.

The gall is composed of parenchyma tissue, mainly pericyclic in origin. In some serial sections pericycle hypertrophy and hyperplasia are evident, suggesting the formation of a side root, but no vascular tissue differentiates in these areas. Extensive pericycle enlargement is already present in some roots 5 days after infection. The cortex also hypertrophies in response to a developing nematode, but the cortex sloughs off in older roots, and the galls observed in mature soybean plants contain little or no cortex tissue.

Associated root abnormalities other than giant cells are typical of plant tumors, and are not described in detail here. In addition to hyperplasia and hypertrophy of pericycle and parenchyma tissue, the following abnormalities have been noted: 1) Abnormal vessels. 2) Areas of necrosis both directly associated with giant cells and more frequently localized near the posterior portions of nematodes. 3) Unusual meristematic activity.

Discussion.—Variations in host-parasite interaction have been described in many ways. It seems useful to separate the host's growth under parasite attack from the parasite's growth. If we consider only 2 classes of host growth and 2 classes of parasite growth, we may construct a 4-place table of terms useful in categorizing hosts.

		Host Growth	
		Good	Poor
Parasite growth	Good	tolerant	susceptible
	Poor	resistant	intolerant

In this paper we have demonstrated that the ability of a soybean host to support growth of root-knot nematodes depends on host-cell response to the presence of the parasite. No attempt has been made to separate tolerant from susceptible hosts or resistant from intolerant hosts. Progress along these lines will depend on the attainment of precise control of both the environment and the intensity of infection. In the present work, no attempt was made to control the numbers of parasites, but all varieties were placed into contact with abundant larvae. The environment was the variable one of a greenhouse without precise light or temperature controls.

Demonstration of a correlation between details of the histopathology of host response and parasite growth rates leaves many questions unanswered. The giant cell obviously has a very different metabolism from that of normal cells. In what way do giant cells of types 2 or 3 differ from those of type 4? And what is the meaning of the excessive inclusions in type 2 giant cells? The peculiar spiral inclusions seen at all stages of type 2 cells react positively for cellulose and pectin and seem related to the excessively heavy deposits of cellulose and pectin on walls of type 4 giant cells. Some fundamental derangement of wall formation seems indicated. Krusberg and Nielsen (7) reported for sweet potato giant cells heavy walls with the same general appearance we observed.

Another problem is the formation of discrete giant cells within a cluster. It seems likely that active cell wall dissolution results from enzymes produced by the host in response to some triggering action by the parasite. The question remains why cell wall dissolution proceeds more rapidly at the ends of growing giant cells than in a radial direction. Since giant cells of more than one type occur within a single cluster, the differences determining final type must be slight.

The peculiar nuclear phenomena need to be elucidated. One unmistakable example of polyploidy occurs in the sections taken 9 days after exposure in a cell very close to a growing giant cell. The nucleus is in metaphase, showing well over 100 chromosomes. The nuclear clusters composed of small pear-shaped nuclei are of great interest. They have been reported in sweet potato infections with root-knot (7), and Němec (9) interpreted them as a stage in the fusion of nuclei. Both the large and small nuclei stain strongly with Feulgen reagent, and the clusters display long Feulgen-positive threads connecting the nuclei together at a center. The significance of two distinct nuclear types is a puzzle.

The resistance of soybeans to root-knot nematodes is expressed in host-cell response to the parasite. In addition, differences in numbers of organisms present in the roots at the time of fixation are notable. Plants of Palmetto soybean have few nematodes, whereas Richland and Adams varieties have many in the sections. The most striking difference between varieties is the frequency of occurrence of type 4 giant cells. Size of nematodes or numbers of eggs can not be accurately correlated with size of giant cells. Deviations of giant cell development from type 4, however, are never associated with rapid female growth and full egg production.

The timed series of sections of suitable (Richland) and unsuitable (S-100) hosts offer significant contrasts. Whereas in soybean variety Richland roots the mitotic activity precedes giant cell growth and extends for some distance longitudinally along the root in both directions beyond the giant cells proper, in variety S-100 roots there is little mitotic activity associated with nematode larvae, and most giant cells are of type 2. Perhaps one aspect of host resistance or intolerance is this failure to respond to larval secretions with hyperplasia, so that a mitotic stimulant or depressant could change a host from one category to another.—Nematology Section, Agricultural Research Service, Beltsville, Maryland; and Cornell University, Department of Plant Pathology, Ornamentals Research Laboratory, Farmingdale, New York.

LITERATURE CITED

1. Baker, K. F. 1957. The U. C. system for producing healthy container-grown plants through the use of clean soil, clean stock, and sanitation. Calif. Univ. Agr. Expt. Sta. Extension Serv. Manual 23. 332 p.

2. Christie, J. R. 1936. The development of root-knot nematode galls. Phytopathology 26:1-22.

3. Dropkin, V. H. 1959. Varietal response of soybeans to Meloidogyne—a bioassay system for separating races of root-knot nematodes. Phytopathology 49:18-23.

4. Dropkin, V. H., and P. E. Nelson. 1959. The pathogenicity of Meloidogyne sp. (root-knot nematodes). Proc. Intern. Congr. Botan. 9th Congr. II:97.

5. Esau, K. 1953. Plant anatomy. John Wiley and Sons, Inc., New York. 735 p.

6. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., Inc. New York. 523 p.

7. Krusberg, L. R., and L. W. Nielsen. 1958. Pathogenesis of root-knot nematodes to the Porto Rico variety of sweet-potato. Phytopathology 48:30-39.

8. Mankau, G. R. 1956. Studies on the host-parasite relationships of Heterodera trifolii (Goffart) Oostenbrink, 1951. Ph.D. thesis, Univ. of Illinois.

9. Němec, B. 1910. Das Problem der Befruchtungsvorgange und andere zytologische Fragen. Berlin. Gebrüder Borntraeger. 1-526. VI. Vielkernige Riesenzellen in Heterodera Gallen. 151-173.

10. Rawlins, T. E., and W. N. Takahashi. 1952. Technics of plant histochemistry and virology. National Press, Millbrae, California. 125 p.

RELATION OF INSECTS TO INTERNAL CORK OF SWEET POTATO IN LOUISIANA

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SUMMARY

Caged and uncaged plants in mother beds and field plantings indicated that the majority of spread of internal cork virus from sweet potato plant to sweet potato plant occurs in field plantings.

Dates-of-planting tests, to determine when most internal cork infection occurred, were made over a 3-year period concurrently with studies of aphid dispersal and occurrence of other insects associated with sweet potatoes. Results related time of maximum field infection with peak flights of cotton aphid, *Aphis gossypii*. No relationship was found with the other insects.

Internal cork was discovered in Louisiana sweet potatoes (10) within a year of its original description as a disease of sweet potato (8). Incidence increased greatly, and by 1952 it was considered a serious threat. Consequently, intensive studies were begun on insect-transmission and spread under field conditions in Louisiana.

Some results have been published: the cotton aphid, *Aphis gossypii* Glover, was demonstrated to be a vector in controlled experiments (4); and field spread from sweet potato to sweet potato gave evidence of transmission as a nonpersistent virus (6). Herein the results of experiments on natural field transmission are correlated with data on insects associated with sweet potato when the plants became infected in the field.

Materials and methods.—Occurrence and prevalence of insects in sweet potato fields in Louisiana were determined in 1955–58. Insects were collected weekly with a sweep net. Records of aphid dispersal were made from tanglefoot traps placed just above the growing vines. Data on aphid dispersal in all major sweet-potato-producing sections of Louisiana will be published separately.

Caged and uncaged plants in mother beds and in field plantings were studied in 1956 to determine the amounts of cork spread. Caged plantings in this and other experiments reported herein were kept under 32×32-mesh Saran screen. In this experiment field plantings were made in the immediate vicinity of mother beds. Plants were bedded February 27, and set in the field June 1. There were 4 replications. Harvest date was November 5, 1956, and the roots were sliced to determine cork incidence March 14, 1957.

Dates-of-planting experiments to determine when most infection occurred were made in St. Landry Parish in 1956 and in East Baton Rouge Parish in 1957 and 1958. Insect prevalence in these plantings was determined by the methods used in the state-wide survey. In the 1956 test, plantings for each of the dates (Table 1) were replicated 4 times in randomized blocks, and the source of inoculum was cork-affected plants in adjacent test plots and surrounding commercial plantings. The 1957 and 1958 tests respectively had 10 and 5 replications, with each succeeding planting adjacent to the previous one. Source of inoculum for the 1957 and 1958 tests was cork-affected plants set in rows alternating with cork-free plantings.

In all experiments internal cork was determined by slicing harvested roots into sections 1/16 in. thick and recording the presence of the characteristic brown-to-black lesions described by Nusbaum (9). No other means of positive identification is known. The source of cork-free plant material was a stock of Unit 1 Porto Rico variety grown 4 years in isolation. Internal cork has never been found in that planting, though the stock is not known to be free of all viruses. Chlorotic spotting and purple-ring spotting on foliage have been commonly observed in the isolated plantings that have produced cork-free roots. Further procedure pertinent to individual experiments is given in the table.

Results and discussion.—The insect survey from sweep net collections revealed that most insect species heavily infesting sweet potato vines in Louisiana fields reached peak populations in late September through October. This was especially true of *Empoasca* spp., the leafhoppers most abundant on sweet potatoes. Species of *Empoasca* taken included *E. abrupta* DeL., *E. bifurcata* DeL., *E. curvexa* Dav. & DeL., *E. erigeron* DeL., *E. fabae* (Harris), *E. fabalis* DeL., *E. recurvata* DeL., *E. sativae* Poos, and *E. solana* DeL. The number of each species apparently depended on location and

Table 1.—Number of dispersing cotton aphids collected on 3 5×6-in. tanglefoot traps placed above sweet potato field plantings, and mean[a] percentages of internal cork in dates-of-planting tests.

Planting date[c]	Aphids collected at 14-day intervals after planting[b]				Total for 120 days	Per cent cork	
	1–14	15–28	29–42	43–56		At harvest	After storage
St. Landry Parish, 1956							
May 1	6	7	16	306	419	1.6	9.3
May 15	7	16	306	76	414	3.7	15.3
June 1	16	306	76	7	407	17.0	23.8
June 15	306	76	7	0	392	3.3	8.0
July 1	76	7	0	1	86	0.8	1.1
LSD at 5%						3.85	8.44
1%						5.40	11.84
L. S. U. Hill farm, Baton Rouge, 1957							
May 6	5	4	4	0	18	1.0	3.7
May 16	5	5	4	2	16	0.8	6.7
May 27	6	2	1	1	13	0.7	1.5
June 5	4	1	2	2	9	1.9	2.2
June 14	4	2	3	1	10	1.4	13.6
June 24	1	2	3	1	7	2.1	9.3
July 3	2	2	1	0	5	0.0	5.5
LSD at 5%						n.s.	6.60
1%						n.s.	8.80
L. S. U. Hill farm, Baton Rouge, 1958							
April 24	3	12	3	6	27	1.7	10.8
May 8	12	3	6	6	22	3.4	7.5
May 22	3	6	6	0	12	1.5	3.2
June 6	6	6	0	1	9	0.3	2.6
June 19	6	0	1	3	24	0.0	5.9
July 3	0	1	3	3	18	1.3	2.9
July 17	1	3	3	0	18	1.0	4.5
LSD at 5%						n.s.	5.47
1%						n.s.	–

[a] Mean of 4, 10, and 5 replications, respectively in 1956, 1957, and 1958 experiments.

[b] For an estimate of actual numbers of aphids dispersing across a 1-acre-wide front 1 ft deep at plant level, multiply numbers of aphids by 336. Numbers of aphids would, however, be somewhat less, since aphids were probably attracted toward the yellow-colored "tree tanglefoot."

[c] The 1957 plantings were at 10-day intervals. Aphids were recorded at 2 week intervals; hence, the overlap of numbers of aphids in the columns by weeks.

date of collection. Other leafhoppers, taken in lesser numbers at infrequent occasions, included *Draeculacephala*, *Homalodisca*, *Graphocephala*, *Graminella*, *Agallia*, *Chlorotettix*, and *Paraphlepsius* species. These appeared somewhat dependent on the presence of weed hosts in sweet potato fields.

Whitefly populations on sweet potatoes increased to large numbers in some localities late in the season of 1956, but were almost totally absent in 1957–58.

The flea beetle, *Chaetocnema confinis* Crotch, and the banded cucumber beetle, *Diabrotica balteata* Lec., were in all areas during the season in each year of the survey. Flea beetles appeared not to leave sweet potato to complete their reproductive cycle on other hosts, as reported by some authors (7).

Over 60 species in 20 genera of dispersing aphids (aphids airborne and adrift to new locations by air movements) were taken from sweet potato fields throughout the state during 3 years of the survey. Most aphids trapped were of the genus *Aphis*. Of these taken during periods of maximum field transmission of internal cork, most were *A. gossypii*. *Myzus persicae* (Sulz.) and *Macrosiphum solanifolii* (Ashm.) were almost completely absent from sweet potato fields when much of the infection took place. Both of these species have been reported to be vectors of internal cork (1, 2, 3, 11). Though *M. persicae* and *M. solanifolii* will colonize sweet potato, they were not found or collected on the traps during much of the growing season. *A. gossypii* was not found to colonize sweet potato in Louisiana, but was collected from traps throughout the growing season. Since nonpersistent viruses depend on rapid transfer from plant to plant, aphids that colonize sweet potato probably would be less efficient vectors than dispersing aphids.

Internal cork was more severe in field plantings than in mother beds. Mean percentage of cork-affected sweet potato roots for treatments in the caged and uncaged plants in mother beds and field plantings experiment was as follows: with plants caged in both mother beds and fields 0%; with mother beds exposed

and field plantings caged 3.3%; with mother beds caged and field plantings exposed 29.9%; and with both mother beds and field plantings exposed 57.9%.

Greatest infection in the field plantings appeared to coincide with aphid flights over sweet potato plants set 2–4 weeks earlier. Plants at that stage had recovered from the shock of transplanting and were characterized by a flush of new growth.

It is well known that viruses are transmitted more readily to young plant tissue (5). Therefore, data from dates-of-planting tests should reveal when field transmission of a virus disease is most abundant. The amount of disease developing in plots, comparable except for dates of planting, can then be correlated with activity of the vector. Table 1 shows relationship of time of aphid flight to infection of sweet potato with internal cork. The data, as shown by aphids collected in aerial traps, relate flights of the cotton aphid, *A. gossypii*, dispersing from cotton and hosts other than sweet potato to periods of maximum field infection.

Large numbers of dispersing cotton aphids were trapped in late June and early July of the 1956 season. Cork lesion development at harvest was greater that year than in subsequent seasons, perhaps because of multiple inoculations, which may result in earlier lesion development. Resulting multiple inoculations appear to have augmented rapid lesion development in the roots. During 1957 and 1958 low numbers of cotton aphids were trapped. Flights were somewhat continuous. Incidence of cork also was low at harvest, but increased considerably after the roots were stored 5 months at 75°F or above (Table 1). When as few as 20 aphids were used to inoculate cork-free plants in a caged test in 1957, only 4% of the roots had cork lesions. From originally cork-affected plants in the same test 52% of the roots were affected. Roots produced the second season from the inoculated stock had cork lesions at harvest comparable to those in roots from originally cork-affected plants (respectively 18% and 27%). It thus appears that root lesion development may be dependent on virus concentration.

Data presented show association of host and vector with spread of internal cork. The effectiveness of cotton aphids in spreading the disease indicates a rapid transfer of virus from plant to plant. Sweet potato is a poor host for the cotton aphid; thus, the aphid feeds lightly on an infected plant and apparently moves to another plant with little delay, thereby transmitting a virus that appears to be nonpersistent in the vector.

Thus far in Louisiana, insects other than cotton aphids have not been proved capable of transmitting any agent contributing to the formation of cork lesions in the roots of sweet potatoes. Studies are in progress to determine if other species of *Aphis* and related genera trapped from brief dispersal flights during infection periods may also contribute to the spread of internal cork. Data presented herein indicate, however, that the efficient spread of internal cork from sweet potato to sweet potato in Louisiana is dependent on cotton aphids, *A. gossypii*, moving from other hosts.—Departments of Entomology and Plant Pathology, Louisiana State University, Baton Rouge.

LITERATURE CITED

1. Hildebrand, E. M. 1958. Two syndromes caused by sweet potato viruses. Science 128:203-204.
2. Hildebrand, E. M., and F. F. Smith. 1956. Aphid transmission of sweet potato cork virus in the greenhouse. (Abstr.) Phytopathology 46:468.
3. Hildebrand, E. M., and F. F. Smith. 1958. Aphid transmission of a virus associated with sweet potato internal cork and masked in feathery-mottle-infected sweet potatoes. U. S. Dept. of Agr. Plant Disease Reptr. 42: 1148-1153.
4. Kantack, E. J., W. J. Martin, and L. D. Newsom. 1958. Transmission of internal cork of sweet potato by the cotton aphid, Aphis gossypii Glover. Science 127:1448.
5. Langeluddeke, P. 1957. Aphids as vectors of virus diseases of potatoes. A literature review. Hofchen-Briefe 10:237-266.
6. Martin, W. J., and E. J. Kantack. 1958. Spread of internal cork of sweet potatoes under field conditions. Louisiana Agr. Expt. Sta. Quart. Rept. 1:12-13.
7. Metcalf, C. L., W. P. Flint, and R. L. Metcalf. 1951. Destructive and useful insects, 3rd edition. McGraw-Hill, New York.
8. Nusbaum, C. J. 1945. A preliminary report on internal cork, a probable virus disease of sweet potato. U. S. Dept. Agr. Plant Disease Reptr. 29:677-678.
9. Nusbaum, C. J. 1946. Internal cork, a new disease of sweet potato of unidentified cause. Phytopathology 36:18-23.
10. Person, L. H., and E. Olson. 1946. The occurrence of internal cork of sweet potatoes in Louisiana. U. S. Dept. Agr. Plant Disease Reptr. 30:477.
11. Rankin, H. W., and J. H. Girardeau, Jr. 1958. Transmission by Myzus persicae (Sulz.) of the internal cork virus of sweet potatoes. U. S. Dept. Agr. Plant Disease Reptr. 42:581-582.

RELATION OF MICROCLIMATES TO THE DEVELOPMENT OF POWDERY MILDEW OF LETTUCE

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SUMMARY

Wet and dry thermocouples were used to measure temperature and RH in small spaces. In a wet field of lettuce RH increased 6% in sunlight and 10% in shade between points 1 m and 5 cm above the surface of the soil; in a dry field of lettuce there was no significant gradient. Gradients in temperature and RH in the sphere of lettuce plants in the field appeared too small to account for gradients in mildew infection on the same plants. Gradients in RH were measured above lettuce leaves in the laboratory. RH was 66% at the leaf surface when room RH was 55%. The percentage of germination of conidia of *Erysiphe cichoracearum* on lettuce leaves in the laboratory, a biological measurement of RH at the leaf surface, indicated that RH at such surfaces was less than 79% (germination below 79% RH is constantly low, at about 2%). Meteorological data indicate that microclimates theoretically could add at most 2 hours to any period favorable for germination of conidia (93-98% RH at 25°C). Since conditions adverse to the formation of microclimates are general in the Salinas Valley, this disease appears to be influenced mainly by macroclimate.

Germination of powdery mildew conidia is greater on leaves of the host than on glass slides at the same relative humidity (RH), according to Longree (5), Yarwood (20), Schnathorst (14), and Weinhold (19). Two explanations have been proposed: 1) the microclimate above the leaves of the host makes RH around conidia more favorable for germination (5), and 2) stimulating chemicals on the leaf surface or emanating from leaves bring about a higher percentage of germination (5, 22). Gradients of temperature and RH between the base and top of plants may account for observed gradients of powdery mildew (*Erysiphe cichoracearum* DC. ex Mérat) on lettuce (*Lactuca sativa* L.) plants (13). The writer indicated in previous papers (13, 15) that the disease gradient was due to physiological gradients in the host, being directly correlated with osmotic values of the leaves.

A study was conducted to determine: 1) whether microclimates exist between the base and the top of lettuce plants in the field, 2) whether microclimatic gradients around the plants account for the gradient of powdery mildew infection, 3) whether microclimates are present at the surface of lettuce leaves, and 4) whether the microclimate at the leaf surface is sufficiently different from the macroclimate to increase the germination of conidia.

Observations and results.—The term *microclimate* refers to climatic conditions in the lowest 2 m of the earth's atmosphere (3). In studies of microclimates in regard to foliar pathogens there are 2 considerations: the climate from the base to the top of plants (designated herein as the plant sphere) and the climate directly above the leaf surface. A microclimate is known to be present in the sphere of plants in certain instances (3), but comparatively few attempts (1, 4, 7, 8, 9, 10, 11, 12, 17, 18, 22) have been made to determine RH directly above the leaves of plants.

A Leeds Northrup 8662 potentiometer with an ice pot as a constant reference temperature was used to take millivolt readings for determining temperature and RH. Thermocouples of 30-gauge (250 μ) duplex copper-constantan wire were made according to the directions of Lorenzen (6). Wet and dry thermocouples were mounted side by side, about 5 mm apart. The wet thermocouple was wrapped with nylon thread, which was moistened with distilled water. The thermocouples were standardized so that both gave the same dry reading under the same conditions. A mechanical microscope-stage mounting (1) aided in making fine adjustments of distance between the tip of the thermocouple and the surface over which the readings were being made; a ring stand with clamp made coarse adjustments possible.

RH was originally determined by exchanging the wire leads of the wet and dry thermocouples at the potentiometer for each determination. This exchange was speeded by inserting a 3-way switch into the circuit. With this method RH readings could be obtained in less than 10 seconds. Accuracy of the apparatus was within 1% RH (the reading was always higher with the thermocouple determination) in the field, and 2% RH (higher with the thermocouple determination) in the laboratory (very little air movement). The error was determined by checking readings obtained thermoelectrically with those obtained with a hand-aspirated psychrometer (Model No. HA/2, Fries Instrument Corporation). A cheesecloth shield was used in the field when shade was desired. All readings were taken in virtually still air (not more than 2 mph) in the field and in the laboratory. This procedure was altered when the effect of air movement on a microclimate above the leaf surface was studied in the greenhouse.

Microclimate in the plant sphere.—Readings of temperature and RH were taken at various heights alongside Great Lakes lettuce plants growing in the field in the Salinas Valley near Spreckels, California. Temperature and RH were determined in direct sunlight and under a cheesecloth shade in a field irrigated 6 days previously. For comparison, readings were taken in a nearby dry field (no water for more than 30 days). The thermocouples were randomly moved up and down at set heights beside lettuce plants, i.e., frame leaves, lower wrapper leaves, and upper wrapper leaves. Readings were also taken 5 cm and 1 m above ground level in the lettuce beds. All readings were alongside plants well within the field. One minute was allowed for temperature equilibrium before a reading was taken. Ten readings were made for each comparison studied.

Table 1.—The microclimatic gradient of relative humidity at various heights above beds of Great Lakes lettuce under various conditions of soil moisture and light.

Field condition [a]	Light condition	Height above plant bed	Av. temperature		Av. RH (%)
			Dry bulb (°C) [c]	Wet bulb (°C) [c]	
Wet [b]	Sunny (artificial shade)	Basal leaves	20.8	16.5	66.0*
		Middle leaves	21.0	16.2	61.5
		Head leaves	21.0	16.1	60.5*
		5 cm	18.8 [d]	15.8	74.0*
		1 m	18.6 [d]	14.4	64.5*
	Sunny	Basal leaves	22.0	16.9	60.6
		Middle leaves	21.7	16.4	58.6
		Head leaves	21.6	16.3	57.6
		5 cm	19.9 [d]	16.0	67.3
		1 m	19.5 [d]	15.0	61.5
Dry [b]	Overcast	Basal leaves	19.0	14.2	59.1
		Middle leaves	19.0	14.0	58.1
		Head leaves	18.9	14.0	58.9
		5 cm	18.8	13.8	57.8
		1 m	18.8	13.6	56.0

[a] Wet and dry field readings were taken on different days.
[b] Wet field was irrigated 6 days previously, dry field not irrigated for 30 days.
[c] Average of 10 determinations.
[d] Readings were taken later in the day than the rest of readings in the series.
* Differences in relative humidity between basal leaves and head leaves, and between the 5-cm and 1-m levels, were statistically significant at the 5% level.

Under cheesecloth shade in the recently irrigated field (Table 1), RH was about 6% higher (statistically significant at the 5% level) near the frame leaves than near the upper wrapper leaves, and about 10% higher (statistically significant at the 5% level) 5 cm above the lettuce beds than at 1 m. A decrease in RH with increasing distance above the beds was also indicated in direct sunlight, though the difference between the lower and upper levels was not statistically significant at the 5% level (Table 1). The dry field had no statistically significant difference in RH between the base and top of lettuce plants or between 5 cm and 1 m above the soil. The plants, nevertheless, showed a marked gradient in powdery mildew infection, i.e., extensive fungus on the lower leaves but much less or none near the top of the plant. In the irrigated field, sun-exposed frame leaves were as much as 2.5°C hotter than shaded frame leaves and RH at the leaf surface was 10% lower.

Microclimate above the leaf surface.—Measurements of microclimatic RH at the leaf surface were made in the laboratory, except for a few determinations in the greenhouse. Following several determinations with thermocouples of RH at various distances above Petri dishes of distilled water, readings were taken down to the upper and lower epidermis of mature basal Great Lakes lettuce leaves from a height of several cm. Illumination and air movement were not conducive to high transpiration (illumination was 200 ft-c at the leaf surface, wind less than 1 mph in the laboratory).

Increase in RH over distilled water was marked as the thermocouples neared the surface. RH increased above leaf surfaces also, but less so than above the water (Fig. 1). Because molecules of water vapor are compressed by the thermocouples, readings taken closer than 1.0 mm above the leaf may show sharp increases in RH (1, 8). Below 1.5 mm, RH increased abruptly in the present study. An apparent error of 14% RH was encountered between 0 and 1.5 mm above lettuce leaves (Fig. 1).

The data were recalculated and plotted (Fig. 2) according to the formula of Frampton and Longree

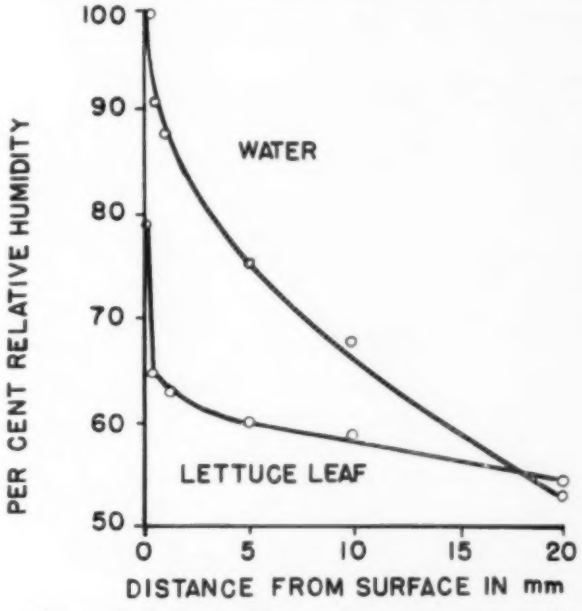

Fig. 1.—Relative humidity in per cent at various distances above the surface of distilled water in a Petri dish and a lettuce leaf in the laboratory. All points on the graph are an average of 3 determinations. Note the abrupt increase in RH starting 0.5 mm above the surface of the lettuce leaf (explained in the text.)

(2), which states that, where R is observed RH at a given distance from the surface of a leaf and S is RH at a large distance from the surface of the leaf, the log (R-S) is proportional to the distance from the leaf. Thus, determining RH at the surface of a leaf requires only the determination of 1 S and 2 R's at 2 arbitrary distances. A straight line was obtained in the above plot (Fig. 2), which agrees with data of Ramsay, Butler, and Sang (8) as plotted by Frampton and Longree (2). When the semi-logarithmic plot of the data for the lettuce leaf was extrapolated from 1.5 mm to 0 mm, a value of about 63% (not 79%, Fig. 1) was obtained for RH at the leaf surface, an increase of 9% over the RH at a large distance from the leaf (RH of the laboratory). When the data for the dish of distilled water were extrapolated from 1.5 mm to 0 mm, a value of 97% RH (theoretically it should be 100%) was obtained for RH at the surface of the water. This indicated an error of 3% RH in the experimental method. Therefore, the real value of RH at the leaf surface under the conditions of this experiment was probably closer to 66 than to 63%.

When similar readings were taken in the greenhouse (illumination 400 ft-c) with considerable air movement from a ventilating fan, no RH gradient was detected above lettuce leaves. Appreciable air movement apparently removed it, as suggested by Ramsay, Butler, and Sang (8).

Percentage of spore germination was tested as a biological means of measuring RH at the leaf surface of Great Lakes lettuce plants in the laboratory. RH in the laboratory averaged 59% at a near-constant temperature of 22°C; illumination was about 400 ft-c at the leaf surface. Air movement was less than 1 mph. As a control, spores were germinated in Petri dishes at 18°C and 100% RH. Germination of conidia on the lettuce leaves, determined by the celloidin strip method (1), averaged 2% in triplicate trials, whereas germination of control spores averaged 63%. The marked difference in germination suggests that RH at the leaf surface was in the range where percentage of germination is low and assumes a nearly constant value, i.e., about 0.1–79.0% (79% was derived by interpolating the graph in Fig. 3 of a previous paper, 14). It thus appears that in no case was RH at the leaf surface greater than 79%. These results agree with data obtained in thermoelectric determinations of RH at the leaf surface. In spite of the low percentage of germination of conidia on lettuce leaves, mildew development was good, probably because of the large spore load used to inoculate the leaves.

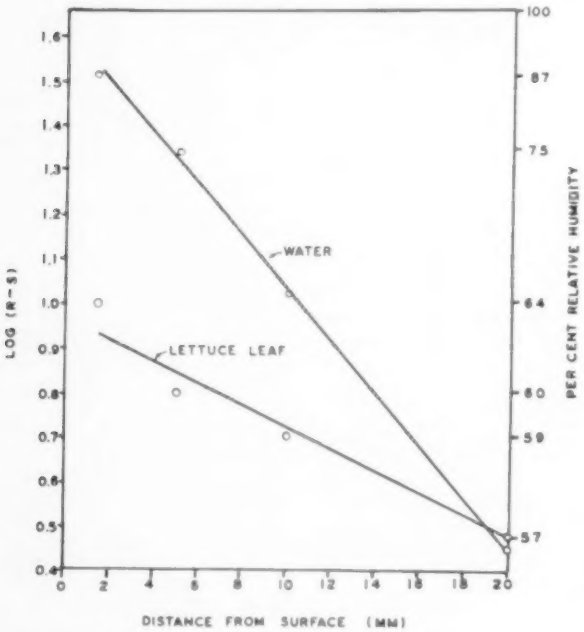

Fig. 2.—Value of log (R-S) plotted against distance from the surface of distilled water and a lettuce leaf where R is RH at a given distance from the surface and S is RH (54%) at a long distance (40 mm) from the surface (see Fig. 1).

Interaction of microclimate and macroclimate.—The present study indicates that a slight microclimate exists in the plant sphere in the field and above the leaf surface in the laboratory under certain conditions. Some of the conditions necessary for the detection of significant microclimates appear to be: 1) little or no air movement, 2) moist field conditions, and 3) shade. Thus, it is estimated that under favorable field conditions RH could possibly be 22% higher at the leaf surface than in the macroclimate (2 m above the ground and up). The figure of 22% RH was arrived at by considering the increase in RH (10%) in the plant sphere and adding to it the increase in RH (12%) at the leaf surface.

An increase of 22% RH at the leaf surface might be of advantage to the fungus in that it could feasibly extend humidity periods favoring germination of conidia (93–98% RH at 25°C, 14). Thus, even though RH of the macroclimate was low the increase in RH at the leaf surface might be high enough to allow germination of a high percentage of powdery mildew conidia during most of any given day.

It was therefore of importance to obtain macroclimatic data in the Salinas Valley. This was done in conjunction with another study, to be reported in another paper.

Fig. 3 shows a typical chart taken from a hygrothermograph located in the Salinas Valley during the height of the mildew season in 1955. It was calculated that the RH of the macroclimate necessary to provide conditions at the leaf surface favoring the germination of most of the germinable conidia would be about 70% (theoretic microclimatic increase would make RH at the leaf surface 92%). In the majority of cases a 70% RH was reached about 1 hour before the maximum RH of the macroclimate of a given day, and it continued for about 1 hour after. Thus, under conditions favorable for formation of a microclimate of over 92% RH, microclimate could extend the period favoring germination of powdery mildew conidia by about 2 hours. On the other hand, conditions essential for the formation of disease-favoring microclimates

are infrequent in the Salinas Valley, mainly because of high wind velocity and high illumination.

Discussion and conclusions.—Differences in temperature and RH between the base and the top of lettuce plants in the field were statistically significant only when the plants were artificially shaded. Based on a study of the effects of temperature and moisture stress on the lettuce powdery mildew fungus (14) the differences in temperature and RH in the plant sphere in the field were not great enough to affect development of this fungus appreciably. It thus appears that gradients of powdery mildew infections on lettuce plants are not explainable on the basis of gradients in temperature and RH in the sphere of the plants. This conclusion lends support to the proposal put forward by the writer in previous papers (13, 15) that the gradient of powdery mildew infection is due to physiological factors. Tapke (16) came to a similar conclusion in studies on the powdery mildew of barley. Similarly, a microclimate at or near the surface of lettuce leaves of the magnitude detected could be of very little if any significance in the development of powdery mildew.

Under conditions of little air movement and low illumination, gradients in RH were detectable both around lettuce plants and above leaves. The Salinas Valley of California, however, where this study was conducted in part, is normally wind-swept. The results of the study on the effects of air movement on the presence of microclimates conducted in the greenhouse indicate that appreciable air movement removes the microclimate above the leaf surface. It is reasonable to assume that wind would also largely remove the very small microclimate in the sphere of lettuce plants in the field as has been shown by others for other plant populations (3).

The only advantage of microclimates in the plant sphere and at the surface of lettuce leaves to the powdery mildew fungus seems to be one of prolonging periods of favorable (high) RH for the germination of conidia. Under the most favorable conditions for the formation of microclimates, i.e., shade, little air movement, and moist field conditions, it is estimated from meteorological data collected in the Salinas Valley (Fig. 3) that this period of high RH would be extended by at most 2 hours of each day. It is therefore concluded that this disease is mainly influenced by the macroclimate.

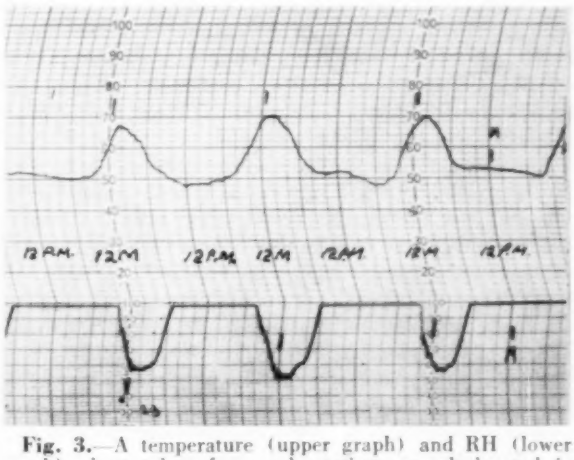

Fig. 3.—A temperature (upper graph) and RH (lower graph) chart taken from a hygrothermograph located in a standard shelter in the Salinas Valley during the summer of 1955.

These studies suggest that the high percentage of germination of conidia of *Sphaerotheca pannosa* (Wallr.) Lév. on rose and peach plants growing under conditions of RH not conducive to germination of conidia, as reported by Longree (5) and Weinhold (19), is due to stimulation of germination by the host rather than due to a microclimate at the leaf surface. This viewpoint is in agreement with that of Yarwood (21) and Yarwood and Hazen (22). Whether the stimulation is chemical, produced by the host, as suggested by Yarwood and Hazen (22), or due to absorption of moisture by the conidia through the epidermis of host leaves, as suggested by Weinhold (19), is not known, but both possibilities appear worthy of investigation.

The RH in plant stands has often been observed to be considerably higher than the RH of the air layer above these stands (3). The increase is related to plant density (3). The increase in lettuce fields may not be as great as it might be in alfalfa fields, for example. Microclimates may be major factors affecting the development of foliar pathogens in dense stands but not in light stands or of only slight importance.—Department of Plant Pathology, University of California, Davis.

LITERATURE CITED

1. Delp, C. J. 1954. Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology 44: 615-626.

2. Frampton, V. L., and Karla Longree. 1941. The vapor pressure gradient above a transpiring leaf. Phytopathology 31:1040-1042.

3. Geiger, R. 1950. The climate near the ground. Translation by M. N. Stewart. Harvard Univ. Press, Cambridge. 482 p.

4. Long, I. F. 1957. Instruments for micro-meteorology. Quart. J. Roy. Meteorol. Soc. (London) 83:202-214.

5. Longree, Karla. 1939. The effect of temperature and relative humidity on the powdery mildew of roses. Cornell Univ. Agr. Expt. Sta. Mem. 223. 43 p.

6. Lorenzen, C., Jr. 1949. The thermocouple in agricultural research. Agr. Eng. 30:275-279.

7. Mellanby, K. 1933. A simple hygrometer for use in small spaces. J. Sci. Instr. 10:349-351.

8. Ramsay, J. A., C. G. Butler, and J. H. Sang. 1938. The humidity gradient at the surface of a transpiring leaf. J. Exptl. Biol. 15:255-265.

9. Rogers, M. N. 1957. Some effects of moisture and of leaf-temperature depression on the development of powdery mildew of roses. (Abstr.) Phytopathology 47:29.

10. Rogers, M. N. 1957. A small electrical hygrometer for microclimatic measurements. (Abstr.) Phytopathology 47: 29.

11. Rogers, M. N. 1957. A small electrical hygrometer for microclimate measurements. Plant Disease Reptr. 41: 897-902.

12. Schnathorst, W. C. 1957. Microclimates and their significance in the development of powdery mildew of lettuce. (Abstr.) Phytopathology 47:533.

13. Schnathorst, W. C. 1959. Resistance in lettuce to

powdery mildew related to osmotic value. Phytopathology 49:562-571.

14. Schnathorst, W. C. 1960. Effects of temperature and moisture stress on the lettuce powdery mildew fungus. Phytopathology 50:304-308.

15. Schnathorst, W. C., and A. R. Weinhold. 1957. An osmotic mechanism for resistance to powdery mildew in lettuce and peach. (Abstr.) Phytopathology 47:533.

16. Tapke, V. F. 1953. Further studies on barley powdery mildew as influenced by environment. Phytopathology 43: 162-166.

17. Thut, H. F. 1938. Relative humidity variations affecting transpiration. Am. J. Botany 25:589-595.

18. Thut, H. F. 1939. The relative humidity gradient of stomatal transpiration. Am. J. Botany 26:315-319.

19. Weinhold, A. 1958. Ecological and physiological studies on the development of peach powdery mildew. Ph.D. thesis, Univ. of Calif.

20. Yarwood, C. E. 1936. The diurnal cycle of the powdery mildew Erysiphe polygoni. J. Agr. Research 52:645-657.

21. Yarwood, C. E. 1959. Microclimate and infection. *In* Plant pathology, problems and progress, 1908-1958: 548-556. The Univ. of Wisconsin Press, Madison, 588 p.

22. Yarwood, C. E., and W. E. Hazen. 1944. The relative humidity at leaf surfaces. Am. J. Botany 31:129-135.

GENETIC CONTROL OF THE HOST-PARASITE RELATIONSHIP IN WHEAT POWDERY MILDEW

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SUMMARY

The relationship between 2 independent, dominant genes conditioning resistance to powdery mildew in wheat and 2 genes conditioning pathogenicity in the *Erysiphe graminis* f. sp. *tritici* was investigated. The 4 possible homozygous host genotypes from a cross involving genes Ml_t and Ml_u were identified by use of 2 differential cultures of *E. graminis* f. sp. *tritici*. These host genotypes were then utilized to identify the 4 possible pathogenic types derived from a cross of the 2 differential cultures. The interactions between host and parasite indicated that for each of the host genes that condition disease reaction there are complementary genes in the pathogen that condition pathogenicity. These genes in the pathogen, which are inherited independently, have been designated as V_t and V_u.

Introduction and review of literature.—Powdery mildew of wheat, caused by *Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal, can be most effectively controlled by using resistant varieties. Much of the research on this disease has therefore dealt with the inheritance of resistance in the host (1, 10, 11). Usually, these studies gave little attention to the role of the pathogen in the disease complex. The more recent studies, however, have been conducted with specific, known races of the pathogen. Carter (1), using 2 races of the pathogen, identified in the host 5 genes that condition resistance. He reported that the variety Normandie, C.I. 12747 (accession number of Cereal Crops Research Branch, Crops Research Division) possesses 2 independent, dominant genes, Ml_t and Ml_u, that condition resistance to these 2 races of *E. graminis* f. sp. *tritici*.

Extensive studies have been carried out on the detection and identification of physiologic races (3, 5, 6, 12, 13). Recent work (8) involving the sexual stage of the pathogen demonstrated great variability in pathogenicity and also pointed out the possibility of genetic control of pathogenicity in the pathogen. The heterothallism of *E. graminis* f. sp. *tritici* (7) greatly facilitates genetic investigations.

Flor (2), working with flax rust, first demonstrated the principle of interrelated genetic systems in the host-pathogen relationship. His studies were concerned with both biological components of the disease complex and utilized information gained regarding one component to assist in the study of the other. He found that for each gene pair conditioning reaction in the host a complementary gene pair in the pathogen conditioned pathogenicity. Twenty-five of these complementary gene interactions were identified in the flax rust complex. Powers and Sando (9) found that the same type of relationship existed between the pathogen *E. graminis* f. sp. *tritici* and its host, *Triticum aestivum* L. They reported the occurrence of 2 genes for pathogenicity in the pathogen complementary to the Ml_t and Ml_u genes in the host. The investigations reported herein, an extension of that work, involve the genetic basis of pathogenicity in the pathogen in relation to the genetic system conditioning reaction in the host.

Materials and methods.—The varieties used were Normandie and Little Club (C.I. 4066). As mentioned, Normandie possesses the genes Ml_t and Ml_u. Normandie was crossed with Little Club, which has the recessive alleles, and F_1, F_2, and F_3 progenies were obtained. The monoascosporic cultures of *E. graminis* f. sp. *tritici* used were 116-2 and 116-3. Culture 116-2 was virulent on varieties of wheat that possess the Ml_u gene, but avirulent on varieties with the Ml_t gene. With culture 116-3, the reverse was true.

The 156 plants in the F_2 progeny from the host cross (Normandie × Little Club) were first inoculated with culture 116-3, and readings were taken after 8–10 days. Susceptible plants were then removed and the remainder were inoculated with culture 116-2. The F_2 plants, except those which did not survive transplanting, were grown to maturity and harvested. The seed from the F_2 plants was planted and the resulting 137 F_3 families were then inoculated with each culture separately. In most cases, 30 plants of each F_3 family were tested with each culture; however, 16 plants were considered an adequate test for the homozygous recessive group. Twenty-four plants were considered sufficient in the segregating and homozygous dominant groups.

Table 1.—Reaction of F_2 wheat plants from the cross Normandie × Little Club wheat to cultures 116-3 and 116-2 of *E. graminis* f. sp. *tritici*.

Culture	No. of F_2 plants resistant	No. of F_2 plants susceptible	χ^2 for 3:1 ratio
116-3	121	35	0.547
116-2	88	33	0.332

Cultures 116-2 and 116-3 were known to be of opposite sexual compatibility types. They were crossed by simultaneously inoculating a susceptible wheat plant with conidia of both cultures and keeping the plant isolated to prevent other mildew infection. The inoculations were made just before the plants headed, and cleistothecia formed within 6 weeks, usually when the plants were maturing. The cleistothecia were the F_1 hybrid of the pathogen cross, and the monoascosporic cultures derived from these fruiting bodies were the F_2 progeny. The details of obtaining these single-spore cultures were reported by Moseman and Powers (4). Each culture in the F_2 progeny was used to inoculate the 4 different homozygous host genotypes involving the Ml_t and Ml_u loci. As a result of the reactions on these hosts, the cultures were classified into pathogenic types. For further substantiation of the classifications, cultures representing the 4 pathogenic types were then intercrossed with each other and with both parental types in all possible combinations. When cleistothecia formed, F_2 cultures were obtained and tested individually on the 4 possible homozygous host genotypes and classified as to pathogenic types. All inoculations were carried out in a growth room held at about 68°F. Fluorescent lighting (cool, white tubes) gave 580 ft-c of light at plant level for 16 hours a day.

Results.—The F_2 plants from the cross Normandie × Little Club wheat were first inoculated with culture 116-3 of *E. graminis* f. sp. *tritici*. As seen in Table 1, 121 were resistant and 35 susceptible to this culture. Of 121 resistant plants inoculated with culture 116-2, 88 were resistant and 33 susceptible. In both instances, the data obtained gave a very good fit for a 3:1 ratio. The results of testing the F_3 families from these F_2 plants with both cultures of the pathogen are shown in Table 2. The data gave a "P" value of 0.75 for the independent assortment of 2 factors and therefore indicated the presence of 2 dominant, independent genes conditioning resistance in the wheat variety Normandie. These findings are in accord with the results of Carter, who designated these genes Ml_t and Ml_u. These host reactions to the 2 cultures of the pathogen permitted the selection of 4 lines representing each of the 4 possible homozygous genotypes involving these 2 gene pairs.

An F_2 population of 78 cultures was obtained from the cross of the 2 cultures of the pathogen. Each of these F_2 cultures was used individually to inoculate plants of the 4 host genotypes mentioned previously. Twenty-seven cultures were capable of infecting all 4 genotypes, which indicated that these cultures possessed both genes conditioning virulence. Twenty cultures could attack only the double recessive genotype, which would be similar to the variety Little Club. These 20 cultures obviously possessed both genes conditioning avirulence. Among the remaining cultures, 19 were similar to the 116-2 parent and 12 similar to 116-3. These 4 pathogenic types clearly represented the 4 possible combinations of 2 genes conditioning virulence. Since the conidial stage of the pathogen used in the inoculations is haploid, it was expected that in the absence of linkage the F_2 generation would contain all possible combinations of the 2 genes in a 1:1:1:1 ratio. The data obtained for this F_2 population fitted this hypothesis with a "P" value of 0.17. These results demonstrated the presence in the pathogen of 2 independent genes that condition pathogenicity.

Table 2.—Reactions of F_3 families from the cross Normandie × Little Club wheat to infection with cultures 116-2 and 116-3 of *E. graminis* f. sp. *tritici*.

	Inoculated with culture 116-2			
Inoculated with culture 116-3	Number susceptible	Number segregating	Number resistant	Totals
No. susceptible	9	15	8	32
No. segregating	11	36	16	63
No. resistant	9	23	10	42
Totals	29	74	34	137[a]

[a] χ^2 for independent assortment of 2 factors = 1.59; "P" value = 0.75.

When cultures representing these 4 pathogenic genotypes were crossed with one another and with the 2 parental cultures in all possible combinations, the results substantiated the findings on the F_2 generation. In the case of each of the crosses, the progenies contained the predicted pathogenic types, and also gave satisfactory "P" values for the predicted ratios (Table 3). Table 3 uses a symbolic designation for the patho-

Table 3.—Segregation observed among progeny from crosses of various pathogenic types of *E. graminis* f. sp. *tritici*.

	Number of each pathogenic genotype in progeny[a]								
	V_tV_u		V_tA_u		A_tV_u		A_tA_u		"P"
Cross	O	C	O	C	O	C	O	C	value
116-2 × 116-3	8	6.25	5	6.25	7	6.25	5	6.25	.78
116-2 × A_tA_u	0	0	0	0	12	10	8	10	.38
116-2 × A_tV_u	0	0	0	0	21	21	0	0	
116-3 × A_tA_u	0	0	8	7.5	0	0	7	7.5	.80
116-3 × V_tV_u	4	6	8	6	0	0	0	0	.25
A_tA_u × A_tA_u (self)	0	0	0	0	0	0	27	27	
A_tA_u × A_tV_u	0	0	0	0	3	4	5	4	.47
A_tA_u × V_tV_u	7	5.25	4	5.25	6	5.25	4	5.25	.75
V_tA_u × A_tV_u	9	7	6	7	8	7	5	7	.70
V_tA_u × V_tV_u	8	7.5	7	7.5	0	0	0	0	.80
A_tV_u × A_tV_u (self)	0	0	0	0	12	12	0	0	
A_tV_u × V_tV_u	10	10	0	0	10	10	0	0	1.00

[a] Symbols V and A indicate virulence and avirulence, respectively, and the subscripts t and u refer to the host gene involved. O and C respectively indicate observed and calculated frequency.

Table 4.—Mildew infection types resulting from the interaction between genetic systems in the host and pathogen.

Genotype of pathogen	Genotype of host			
	$Ml_tMl_tMl_uMl_u$ [a]	$Ml_tMl_tml_uml_u$	$ml_tml_tMl_uMl_u$	$ml_tml_tml_uml_u$ [a]
V_tV_u	S [c]	S	S	S
V_tA_u [b]	R	S	R	S
A_tV_u [b]	R	R	S	S
A_tA_u	R	R	R	S

[a] Parental types of host cross.
[b] Parental types of pathogen cross.
[c] S and R respectively indicate susceptible and resistant types.

genic genotypes of the various cultures crossed. The symbols V and A were used to designate virulence and avirulence, respectively, and the subscripts $_t$ and $_u$ to indicate the particular host gene involved.

Discussion.—The results clearly demonstrate that the wheat variety Normandie possesses the genes Ml_t and Ml_u, which condition a resistant reaction to *E. graminis* f. sp. *tritici*. Moreover, studies of the mildew cultures 116-2 and 116-3 revealed 2 independent genes for pathogenicity that were complementary to the 2 genes in the host. The alleles for virulence have been designated V_t and V_u, and the alleles for avirulence A_t and A_u. The genotype of culture 116-2 can therefore be described as A_tV_u and that of 116-3 as V_tA_u.

Table 4 gives the infection type produced by each combination of host and pathogen genotype. The infection types are determined by the complementary interaction of single genes in the pathogen (for either virulence or avirulence) and in the host (for either resistance or susceptibility). Of the 16 possible combinations between the 4 genotypes of the host and the 4 of the pathogen, 7 result in resistant infection types. Resistant type infections are found only when at least one gene for avirulence occurs in the pathogen and a complementary gene pair for resistance occurs in the host. All other combinations result in infections of a susceptible type. This indicates that interactions conditioning a resistant infection type were expressed regardless of other gene combinations that otherwise would have conditioned susceptibility.

These results also indicate a definite gene-for-gene relationship between the genes Ml_t and V_t and between Ml_u and V_u. In regard to the genes investigated in the present study, it can be stated that for each gene conditioning response in the host, there is a complementary gene conditioning pathogenicity in the pathogen. If it can be assumed that this relationship exists between other genes for response in the host and for pathogenicity in the pathogen, then it should be possible to develop host lines with other combinations of known genes conditioning response. Likewise, cultures of *E. graminis* f. sp. *tritici* with known combinations of genes conditioning pathogenicity can be developed. These cultures should greatly facilitate the further study of the genetic control of host-pathogen interactions since once the genotype of one component of a host-pathogen complex is determined, it greatly assists in the genetic study of the other component.

A series of host lines with known genes conditioning reaction, both singly and in all combinations, could be developed and would be invaluable in the study of pathogenicity in the parasite. New pathogenic races could be more easily recognized and their genes conditioning pathogenicity identified. Conversely, a series of monogenic cultures of the pathogen, each with a specific gene conditioning virulence, would greatly facilitate the identification of genes conditioning resistance in the host. If a variety should be resistant to the entire monogenic series of cultures, then it could be assumed either that the variety possessed a gene conditioning resistance that was not complementary to any of the genes conditioning virulence in the series or that it possessed a combination of these genes. Further inoculations with cultures having different combinations of genes conditioning virulence would give additional information as to the genes conditioning resistance in the host variety.

This method of utilizing one component of the host-pathogen complex to gain information regarding the other also offers advantages in the field of plant breeding. In regard to the host, genes conditioning reaction in a series of varieties could be catalogued and used singly or in any combination, depending on the pathogenic genotype of the prevalent races of the pathogen. In addition, a series of cultures of the pathogen with known genotypes could be used to great advantage in evaluating the progeny from various genetic studies.—Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

LITERATURE CITED

1. Carter, M. V. 1954. Additional genes in Triticum vulgare for resistance to Erysiphe graminis tritici. Australian J. Biol. Sci. 7:411-414.

2. Flor, H. H. 1955. Host-parasite interaction in flax rust—its genetics and other implications. Phytopathology 45: 680-685.

3. Lowther, C. V. 1950. Pathogenicity of physiologic races of Erysiphe graminis tritici in the Southeastern United States. (Abstr.) Phytopathology 40:872.

4. Moseman, J. G., and H. R. Powers, Jr. 1957. Function and longevity of cleistothecia of Erysiphe graminis f. sp. hordei. Phytopathology 47:53-56.

5. Newton, Margaret, and W. J. Cherewick. 1947. Erysiphe graminis in Canada. Can. J. Research C. 25:73-93.

6. Nover, I. 1957. Sechsjährige Beobachtungen über die physiologische Spezialisierung des echten Mehltaus (Erysiphe graminis DC.) von Weizen und Gerste in Deutschland. Phytopathol. Z. 31:85-107.

7. Powers, H. R., Jr., and J. G. Moseman. 1956. Heterothallism in Erysiphe graminis tritici. (Abstr.) Phytopathology 46:23.

8. Powers, H. R., Jr., and J. G. Moseman. 1957. Pathogenic variability within cleistothecia of Erysiphe graminis. Phytopathology 47:136-138.

9. Powers, H. R., Jr., and W. J. Sando. 1957. Genetics of host-parasite relationship in powdery mildew of wheat. (Abstr.) Phytopathology 47:453.

10. Pugsley, A. T., and M. V. Carter. 1953. The resistance of twelve varieties of Triticum vulgare to Erysiphe graminis tritici. Australian J. Biol. Sci. 6:335-346.

11. Ray, D. A., T. T. Hebert, and G. K. Middleton. 1954. Inheritance of resistance to powdery mildew in wheat. Agron. J. 46:379-383.

12. Taylor, J. W., H. A. Rodenhiser, and B. B. Bayles. 1949. Physiologic races of Erysiphe graminis tritici in the Southeastern United States. Agron. J. 41:134-135.

13. Vallega, J., and H. Cenoz. 1943. Reaccion de algunos trigos a las razas fisiologicas de Erysiphe graminis tritici comunes en Argentina. Ann. Inst. Fitotec. S. Catalina 3: 45-48.

REDUCTION IN PATHOGENICITY AND TOXIN PRODUCTION IN DISEASED HELMINTHOSPORIUM VICTORIAE

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SUMMARY

The pathogenicity of diseased and normal cultures of *Helminthosporium victoriae* was compared by 2 methods of inoculation of susceptible oats. The number of plants surviving inoculation of the primary leaf and infestation of soil indicated that diseased *H. victoriae* was significantly less pathogenic than normal *H. victoriae*. Normal and diseased *H. victoriae* combined and added to soil, showed greater pathogenicity than diseased *H. victoriae* but less than the normal fungus. When susceptible oats were seeded 30 days after infestation of the soil, normal *H. victoriae* remained highly pathogenic but the pathogenicity of normal and diseased *H. victoriae* in combination approached that of the diseased *H. victoriae*. Mycelial growth that developed from single conidia isolated from diseased *H. victoriae* of the isolate D-1 was always normal. This precluded comparative pathogenicity studies of inoculation with conidial suspensions. At 24 or 28°C the growth and toxin production on a modified Fries' medium at 7, 14, 21, and 28 days were greater for normal than for diseased *H. victoriae*. Both diseased and normal *H. victoriae* grew well at 32°C, but toxin production was very low.

Some of the characteristics of a transmissible disease of the fungus *Helminthosporium victoriae* Meehan & Murphy have been described (2). The symptoms consisted of almost complete inhibition of growth of the advancing margins of affected colonies, and collapse or lysis of the existing aerial mycelium. The fungus in these abnormal colonies was not killed, but there was a marked inability of the fungus to continue growth. Therefore it was considered of interest to compare the pathogenicity of the diseased fungus with that of normal *H. victoriae*. A second purpose of the investigation was to explore the possibility of biological control of a pathogenic fungus.

Materials and methods.—The pathogenicity studies were made with a single isolate of *H. victoriae* (normal) and its diseased homolog, herein respectively designated N-1 and D-1. The inocula of diseased and normal *H. victoriae* for all pathogenicity studies were increased on potato-dextrose agar (PDA). Normal *H. victoriae* was increased simply by subculturing from normal colonies. Growth of the diseased fungus by subculturing from diseased colonies was far too limited; therefore the diseased inoculum was prepared by plating 3-mm cubes of normal *H. victoriae* plus agar (4 per plate) that had been dipped into a macerated, clarified suspension of diseased *H. victoriae*. Thus the diseased and normal inoculum for each experiment always originated from the same culture plate of normal *H. victoriae*. The preparation of diseased or abnormal juice has been described (2).

After growth of the cultures for 10 days at room temperature the inoculum was prepared by fragmenting 15 culture plates of mycelium plus agar of normal or diseased *H. victoriae* in 2500 ml of water in a Waring blender. Inoculum containing both normal and diseased *H. victoriae* in combination consisted of 15 plates of each type fragmented in 2500 ml of water.

The susceptible oat variety Victorgrain 48-93 was used throughout. All pathogenicity and disease development studies were made in the greenhouse during the cooler months, thereby closely paralleling the growing season of oats in Louisiana.

In the studies dealing with growth and toxin production, diseased and normal *H. victoriae* were grown on 50 ml of a modified Fries' medium (4) in 500-ml flasks. Bioassays of the toxin and determinations for units of toxin per ml were made according to the methods described by Luke and Wheeler (4).

Results.—*Single-conidial isolations from diseased colonies.*—Spore counts from diseased and normal colonies of *H. victoriae* of the respective isolates D-1 and N-1 1, 2, and 3 weeks after plating have shown that spore production by diseased colonies was less than 1% of that of normal colonies. Conidia from diseased colonies were streaked on the surface of 4% water agar and picked individually with fine glass needles. The conidia were transferred to plates of PDA and incubated at room temperature. The colonies that developed showed none of the symptoms of diseased growth, and subcultures from such colonies always produced normal growth. This was not the case, however, with all isolates of the fungus. Some of the conidia from diseased colonies of the isolate D-6 yielded diseased cultures, whereas others yielded ap-

powdery mildew related to osmotic value. Phytopathology 49:562-571.

14. Schnathorst, W. C. 1960. Effects of temperature and moisture stress on the lettuce powdery mildew fungus. Phytopathology 50:304-308.

15. Schnathorst, W. C., and A. R. Weinhold. 1957. An osmotic mechanism for resistance to powdery mildew in lettuce and peach. (Abstr.) Phytopathology 47:533.

16. Tapke, V. F. 1953. Further studies on barley powdery mildew as influenced by environment. Phytopathology 43: 162-166.

17. Thut, H. F. 1938. Relative humidity variations affecting transpiration. Am. J. Botany 25:589-595.

18. Thut, H. F. 1939. The relative humidity gradient of stomatal transpiration. Am. J. Botany 26:315-319.

19. Weinhold, A. 1958. Ecological and physiological studies on the development of peach powdery mildew. Ph.D. thesis, Univ. of Calif.

20. Yarwood, C. E. 1936. The diurnal cycle of the powdery mildew Erysiphe polygoni. J. Agr. Research 52:645-657.

21. Yarwood, C. E. 1959. Microclimate and infection. *In* Plant pathology, problems and progress, 1908-1958: 548-556. The Univ. of Wisconsin Press, Madison, 588 p.

22. Yarwood, C. E., and W. E. Hazen. 1944. The relative humidity at leaf surfaces. Am. J. Botany 31:129-135.

GENETIC CONTROL OF THE HOST-PARASITE RELATIONSHIP IN WHEAT POWDERY MILDEW

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SUMMARY

The relationship between 2 independent, dominant genes conditioning resistance to powdery mildew in wheat and 2 genes conditioning pathogenicity in the *Erysiphe graminis* f. sp. *tritici* was investigated. The 4 possible homozygous host genotypes from a cross involving genes Ml_t and Ml_u were identified by use of 2 differential cultures of *E. graminis* f. sp. *tritici*. These host genotypes were then utilized to identify the 4 possible pathogenic types derived from a cross of the 2 differential cultures. The interactions between host and parasite indicated that for each of the host genes that condition disease reaction there are complementary genes in the pathogen that condition pathogenicity. These genes in the pathogen, which are inherited independently, have been designated as V_t and V_u.

Introduction and review of literature.—Powdery mildew of wheat, caused by *Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal, can be most effectively controlled by using resistant varieties. Much of the research on this disease has therefore dealt with the inheritance of resistance in the host (1, 10, 11). Usually, these studies gave little attention to the role of the pathogen in the disease complex. The more recent studies, however, have been conducted with specific, known races of the pathogen. Carter (1), using 2 races of the pathogen, identified in the host 5 genes that condition resistance. He reported that the variety Normandie, C.I. 12747 (accession number of Cereal Crops Research Branch, Crops Research Division) possesses 2 independent, dominant genes, Ml_t and Ml_u, that condition resistance to these 2 races of *E. graminis* f. sp. *tritici*.

Extensive studies have been carried out on the detection and identification of physiologic races (3, 5, 6, 12, 13). Recent work (8) involving the sexual stage of the pathogen demonstrated great variability in pathogenicity and also pointed out the possibility of genetic control of pathogenicity in the pathogen. The heterothallism of *E. graminis* f. sp. *tritici* (7) greatly facilitates genetic investigations.

Flor (2), working with flax rust, first demonstrated the principle of interrelated genetic systems in the host-pathogen relationship. His studies were concerned with both biological components of the disease complex and utilized information gained regarding one component to assist in the study of the other. He found that for each gene pair conditioning reaction in the host a complementary gene pair in the pathogen conditioned pathogenicity. Twenty-five of these complementary gene interactions were identified in the flax rust complex. Powers and Sando (9) found that the same type of relationship existed between the pathogen *E. graminis* f. sp. *tritici* and its host, *Triticum aestivum* L. They reported the occurrence of 2 genes for pathogenicity in the pathogen complementary to the Ml_t and Ml_u genes in the host. The investigations reported herein, an extension of that work, involve the genetic basis of pathogenicity in the pathogen in relation to the genetic system conditioning reaction in the host.

Materials and methods.—The varieties used were Normandie and Little Club (C.I. 4066). As mentioned, Normandie possesses the genes Ml_t and Ml_u. Normandie was crossed with Little Club, which has the recessive alleles, and F_1, F_2, and F_3 progenies were obtained. The monoascosporic cultures of *E. graminis* f. sp. *tritici* used were 116-2 and 116-3. Culture 116-2 was virulent on varieties of wheat that possess the Ml_u gene, but avirulent on varieties with the Ml_t gene. With culture 116-3, the reverse was true.

The 156 plants in the F_2 progeny from the host cross (Normandie $\times$ Little Club) were first inoculated with culture 116-3, and readings were taken after 8–10 days. Susceptible plants were then removed and the remainder were inoculated with culture 116-2. The F_2 plants, except those which did not survive transplanting, were grown to maturity and harvested. The seed from the F_2 plants was planted and the resulting 137 F_3 families were then inoculated with each culture separately. In most cases, 30 plants of each F_3 family were tested with each culture; however, 16 plants were considered an adequate test for the homozygous recessive group. Twenty-four plants were considered sufficient in the segregating and homozygous dominant groups.

Table 1.—Reaction of F_2 wheat plants from the cross Normandie × Little Club wheat to cultures 116-3 and 116-2 of *E. graminis* f. sp. *tritici*.

Culture	No. of F_2 plants resistant	No. of F_2 plants susceptible	χ^2 for 3:1 ratio
116-3	121	35	0.547
116-2	88	33	0.332

Cultures 116-2 and 116-3 were known to be of opposite sexual compatibility types. They were crossed by simultaneously inoculating a susceptible wheat plant with conidia of both cultures and keeping the plant isolated to prevent other mildew infection. The inoculations were made just before the plants headed, and cleistothecia formed within 6 weeks, usually when the plants were maturing. The cleistothecia were the F_1 hybrid of the pathogen cross, and the monoascosporic cultures derived from these fruiting bodies were the F_2 progeny. The details of obtaining these single-spore cultures were reported by Moseman and Powers (4). Each culture in the F_2 progeny was used to inoculate the 4 different homozygous host genotypes involving the Ml_t and Ml_u loci. As a result of the reactions on these hosts, the cultures were classified into pathogenic types. For further substantiation of the classifications, cultures representing the 4 pathogenic types were then intercrossed with each other and with both parental types in all possible combinations. When cleistothecia formed, F_2 cultures were obtained and tested individually on the 4 possible homozygous host genotypes and classified as to pathogenic types. All inoculations were carried out in a growth room held at about 68°F. Fluorescent lighting (cool, white tubes) gave 580 ft-c of light at plant level for 16 hours a day.

Results.—The F_2 plants from the cross Normandie × Little Club wheat were first inoculated with culture 116-3 of *E. graminis* f. sp. *tritici*. As seen in Table 1, 121 were resistant and 35 susceptible to this culture. Of 121 resistant plants inoculated with culture 116-2, 88 were resistant and 33 susceptible. In both instances, the data obtained gave a very good fit for a 3:1 ratio. The results of testing the F_3 families from these F_2 plants with both cultures of the pathogen are shown in Table 2. The data gave a "P" value of 0.75 for the independent assortment of 2 factors and therefore indicated the presence of 2 dominant, independent genes conditioning resistance in the wheat variety Normandie. These findings are in accord with the results of Carter, who designated these genes Ml_t and Ml_u. These host reactions to the 2 cultures of the pathogen permitted the selection of 4 lines representing each of the 4 possible homozygous genotypes involving these 2 gene pairs.

An F_2 population of 78 cultures was obtained from the cross of the 2 cultures of the pathogen. Each of these F_2 cultures was used individually to inoculate plants of the 4 host genotypes mentioned previously. Twenty-seven cultures were capable of infecting all 4 genotypes, which indicated that these cultures possessed both genes conditioning virulence. Twenty cultures could attack only the double recessive genotype, which would be similar to the variety Little Club. These 20 cultures obviously possessed both genes conditioning avirulence. Among the remaining cultures, 19 were similar to the 116-2 parent and 12 similar to 116-3. These 4 pathogenic types clearly represented the 4 possible combinations of 2 genes conditioning virulence. Since the conidial stage of the pathogen used in the inoculations is haploid, it was expected that in the absence of linkage the F_2 generation would contain all possible combinations of the 2 genes in a 1:1:1:1 ratio. The data obtained for this F_2 population fitted this hypothesis with a "P" value of 0.17. These results demonstrated the presence in the pathogen of 2 independent genes that condition pathogenicity.

Table 2.—Reactions of F_2 families from the cross Normandie × Little Club wheat to infection with cultures 116-2 and 116-3 of *E. graminis* f. sp. *tritici*.

Inoculated with culture 116-3	Inoculated with culture 116-2: Number susceptible	Number segregating	Number resistant	Totals
No. susceptible	9	15	8	32
No. segregating	11	36	16	63
No. resistant	9	23	10	42
Totals	29	74	34	137 [a]

[a] χ^2 for independent assortment of 2 factors = 1.59; "P" value = 0.75.

When cultures representing these 4 pathogenic genotypes were crossed with one another and with the 2 parental cultures in all possible combinations, the results substantiated the findings on the F_2 generation. In the case of each of the crosses, the progenies contained the predicted pathogenic types, and also gave satisfactory "P" values for the predicted ratios (Table 3). Table 3 uses a symbolic designation for the patho-

Table 3.—Segregation observed among progeny from crosses of various pathogenic types of *E. graminis* f. sp. *tritici*.

Cross	Number of each pathogenic genotype in progeny [a]: V_tV_u O	C	V_tA_u O	C	A_tV_u O	C	A_tA_u O	C	"P" value
116-2 × 116-3	8	6.25	5	6.25	7	6.25	5	6.25	.78
116-2 × A_tA_u	0	0	0	0	12	10	8	10	.38
116-2 × A_tV_u	0	0	0	0	21	21	0	0	
116-3 × A_tA_u	0	0	8	7.5	0	0	7	7.5	.80
116-3 × V_tV_u	4	6	8	6	0	0	0	0	.25
A_tA_u × A_tA_u (self)	0	0	0	0	0	0	27	27	
A_tA_u × A_tV_u	0	0	0	0	3	4	5	4	.47
A_tA_u × V_tV_u	7	5.25	4	5.25	6	5.25	4	5.25	.75
V_tA_u × A_tV_u	9	7	6	7	8	7	5	7	.70
V_tA_u × V_tV_u	8	7.5	7	7.5	0	0	0	0	.80
A_tV_u × A_tV_u (self)	0	0	0	0	12	12	0	0	
A_tV_u × V_tV_u	10	10	0	0	10	10	0	0	1.00

[a] Symbols V and A indicate virulence and avirulence, respectively, and the subscripts t and u refer to the host gene involved. O and C respectively indicate observed and calculated frequency.

Table 4.—Mildew infection types resulting from the interaction between genetic systems in the host and pathogen.

Genotype of pathogen	Genotype of host			
	$Ml_tMl_tMl_uMl_u$ [a]	$Ml_tMl_tml_uml_u$	$ml_tml_tMl_uMl_u$	$ml_tml_tml_uml_u$ [a]
V_tV_u	S [c]	S	S	S
V_tA_u [b]	R	S	R	S
A_tV_u [b]	R	R	S	S
A_tA_u	R	R	R	S

[a] Parental types of host cross.
[b] Parental types of pathogen cross.
[c] S and R respectively indicate susceptible and resistant types.

genic genotypes of the various cultures crossed. The symbols V and A were used to designate virulence and avirulence, respectively, and the subscripts $_t$ and $_u$ to indicate the particular host gene involved.

Discussion.—The results clearly demonstrate that the wheat variety Normandie possesses the genes Ml_t and Ml_u, which condition a resistant reaction to *E. graminis* f. sp. *tritici*. Moreover, studies of the mildew cultures 116-2 and 116-3 revealed 2 independent genes for pathogenicity that were complementary to the 2 genes in the host. The alleles for virulence have been designated V_t and V_u, and the alleles for avirulence A_t and A_u. The genotype of culture 116-2 can therefore be described as A_tV_u and that of 116-3 as V_tA_u.

Table 4 gives the infection type produced by each combination of host and pathogen genotype. The infection types are determined by the complementary interaction of single genes in the pathogen (for either virulence or avirulence) and in the host (for either resistance or susceptibility). Of the 16 possible combinations between the 4 genotypes of the host and the 4 of the pathogen, 7 result in resistant infection types. Resistant type infections are found only when at least one gene for avirulence occurs in the pathogen and a complementary gene pair for resistance occurs in the host. All other combinations result in infections of a susceptible type. This indicates that interactions conditioning a resistant infection type were expressed regardless of other gene combinations that otherwise would have conditioned susceptibility.

These results also indicate a definite gene-for-gene relationship between the genes Ml_t and V_t and between Ml_u and V_u. In regard to the genes investigated in the present study, it can be stated that for each gene conditioning response in the host, there is a complementary gene conditioning pathogenicity in the pathogen. If it can be assumed that this relationship exists between other genes for response in the host and for pathogenicity in the pathogen, then it should be possible to develop host lines with other combinations of known genes conditioning response. Likewise, cultures of *E. graminis* f. sp. *tritici* with known combinations of genes conditioning pathogenicity can be developed. These cultures should greatly facilitate the further study of the genetic control of host-pathogen interactions since once the genotype of one component of a host-pathogen complex is determined, it greatly assists in the genetic study of the other component.

A series of host lines with known genes conditioning reaction, both singly and in all combinations, could be developed and would be invaluable in the study of pathogenicity in the parasite. New pathogenic races could be more easily recognized and their genes conditioning pathogenicity identified. Conversely, a series of monogenic cultures of the pathogen, each with a specific gene conditioning virulence, would greatly facilitate the identification of genes conditioning resistance in the host. If a variety should be resistant to the entire monogenic series of cultures, then it could be assumed either that the variety possessed a gene conditioning resistance that was not complementary to any of the genes conditioning virulence in the series or that it possessed a combination of these genes. Further inoculations with cultures having different combinations of genes conditioning virulence would give additional information as to the genes conditioning resistance in the host variety.

This method of utilizing one component of the host-pathogen complex to gain information regarding the other also offers advantages in the field of plant breeding. In regard to the host, genes conditioning reaction in a series of varieties could be catalogued and used singly or in any combination, depending on the pathogenic genotype of the prevalent races of the pathogen. In addition, a series of cultures of the pathogen with known genotypes could be used to great advantage in evaluating the progeny from various genetic studies.—Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

LITERATURE CITED

1. Carter, M. V. 1954. Additional genes in Triticum vulgare for resistance to Erysiphe graminis tritici. Australian J. Biol. Sci. 7:411-414.

2. Flor, H. H. 1955. Host-parasite interaction in flax rust—its genetics and other implications. Phytopathology 45: 680-685.

3. Lowther, C. V. 1950. Pathogenicity of physiologic races of Erysiphe graminis tritici in the Southeastern United States. (Abstr.) Phytopathology 40:872.

4. Moseman, J. G., and H. R. Powers, Jr. 1957. Function and longevity of cleistothecia of Erysiphe graminis f. sp. hordei. Phytopathology 47:53-56.

5. Newton, Margaret, and W. J. Cherewick. 1947. Erysiphe graminis in Canada. Can. J. Research C. 25:73-93.

6. Nover, I. 1957. Sechsjährige Beobachtungen über die physiologische Spezialisierung des echten Mehltaus (Erysiphe graminis DC.) von Weizen und Gerste in Deutschland. Phytopathol. Z. 31:85-107.

7. Powers, H. R., Jr., and J. G. Moseman. 1956. Heterothallism in Erysiphe graminis tritici. (Abstr.) Phytopathology 46:23.

8. Powers, H. R., Jr., and J. G. Moseman. 1957. Pathogenic variability within cleistothecia of Erysiphe graminis. Phytopathology 47:136-138.

9. Powers, H. R., Jr., and W. J. Sando. 1957. Genetics of host-parasite relationship in powdery mildew of wheat. (Abstr.) Phytopathology 47:453.

10. Pugsley, A. T., and M. V. Carter. 1953. The resistance of twelve varieties of Triticum vulgare to Erysiphe graminis tritici. Australian J. Biol. Sci. 6:335-346.

11. Ray, D. A., T. T. Hebert, and G. K. Middleton. 1954. Inheritance of resistance to powdery mildew in wheat. Agron. J. 46:379-383.

12. Taylor, J. W., H. A. Rodenhiser, and B. B. Bayles. 1949. Physiologic races of Erysiphe graminis tritici in the Southeastern United States. Agron. J. 41:134-135.

13. Vallega, J., and H. Cenoz. 1943. Reaccion de algunos trigos a las razas fisiologicas de Erysiphe graminis tritici comunes en Argentina. Ann. Inst. Fitotec. S. Catalina 3: 45-48.

REDUCTION IN PATHOGENICITY AND TOXIN PRODUCTION IN DISEASED HELMINTHOSPORIUM VICTORIAE

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SUMMARY

The pathogenicity of diseased and normal cultures of *Helminthosporium victoriae* was compared by 2 methods of inoculation of susceptible oats. The number of plants surviving inoculation of the primary leaf and infestation of soil indicated that diseased *H. victoriae* was significantly less pathogenic than normal *H. victoriae*. Normal and diseased *H. victoriae* combined and added to soil, showed greater pathogenicity than diseased *H. victoriae* but less than the normal fungus. When susceptible oats were seeded 30 days after infestation of the soil, normal *H. victoriae* remained highly pathogenic but the pathogenicity of normal and diseased *H. victoriae* in combination approached that of the diseased *H. victoriae*. Mycelial growth that developed from single conidia isolated from diseased *H. victoriae* of the isolate D-1 was always normal. This precluded comparative pathogenicity studies of inoculation with conidial suspensions. At 24 or 28°C the growth and toxin production on a modified Fries' medium at 7, 14, 21, and 28 days were greater for normal than for diseased *H. victoriae*. Both diseased and normal *H. victoriae* grew well at 32°C, but toxin production was very low.

Some of the characteristics of a transmissible disease of the fungus *Helminthosporium victoriae* Meehan & Murphy have been described (2). The symptoms consisted of almost complete inhibition of growth of the advancing margins of affected colonies, and collapse or lysis of the existing aerial mycelium. The fungus in these abnormal colonies was not killed, but there was a marked inability of the fungus to continue growth. Therefore it was considered of interest to compare the pathogenicity of the diseased fungus with that of normal *H. victoriae*. A second purpose of the investigation was to explore the possibility of biological control of a pathogenic fungus.

Materials and methods.—The pathogenicity studies were made with a single isolate of *H. victoriae* (normal) and its diseased homolog, herein respectively designated N-1 and D-1. The inocula of diseased and normal *H. victoriae* for all pathogenicity studies were increased on potato-dextrose agar (PDA). Normal *H. victoriae* was increased simply by subculturing from normal colonies. Growth of the diseased fungus by subculturing from diseased colonies was far too limited; therefore the diseased inoculum was prepared by plating 3-mm cubes of normal *H. victoriae* plus agar (4 per plate) that had been dipped into a macerated, clarified suspension of diseased *H. victoriae*. Thus the diseased and normal inoculum for each experiment always originated from the same culture plate of normal *H. victoriae*. The preparation of diseased or abnormal juice has been described (2).

After growth of the cultures for 10 days at room temperature the inoculum was prepared by fragmenting 15 culture plates of mycelium plus agar of normal or diseased *H. victoriae* in 2500 ml of water in a Waring blender. Inoculum containing both normal and diseased *H. victoriae* in combination consisted of 15 plates of each type fragmented in 2500 ml of water.

The susceptible oat variety Victorgrain 48-93 was used throughout. All pathogenicity and disease development studies were made in the greenhouse during the cooler months, thereby closely paralleling the growing season of oats in Louisiana.

In the studies dealing with growth and toxin production, diseased and normal *H. victoriae* were grown on 50 ml of a modified Fries' medium (4) in 500-ml flasks. Bioassays of the toxin and determinations for units of toxin per ml were made according to the methods described by Luke and Wheeler (4).

Results.—*Single-conidial isolations from diseased colonies.*—Spore counts from diseased and normal colonies of *H. victoriae* of the respective isolates D-1 and N-1 1, 2, and 3 weeks after plating have shown that spore production by diseased colonies was less than 1% of that of normal colonies. Conidia from diseased colonies were streaked on the surface of 4% water agar and picked individually with fine glass needles. The conidia were transferred to plates of PDA and incubated at room temperature. The colonies that developed showed none of the symptoms of diseased growth, and subcultures from such colonies always produced normal growth. This was not the case, however, with all isolates of the fungus. Some of the conidia from diseased colonies of the isolate D-6 yielded diseased cultures, whereas others yielded ap-

parently normal cultures. Subcultures from the latter, however, always resulted in diseased growth. Consequently, it was not possible to compare the pathogenicity of diseased and normal *H. victoriae* by spraying conidia on susceptible oats.

The development of normal growth from conidia of the isolate D-1 indicated that the agent responsible for the abnormality in *H. victoriae* was not present in such conidia. An earlier study (2) demonstrated that the disease-inciting agent of *H. victoriae* was intimately associated with diseased mycelium, and not present in the medium supporting diseased growth, or, if present, not capable of infecting normal mycelium independent of viable fragments of the diseased fungus. It has also been found that subcultures from diseased colonies, though always diseased, often developed sectors of rapid, normal growth. Such sectors may have arisen from the germination of conidia or from an unaffected strand of mycelium. The sectors of normal *H. victoriae* were not resistant to the agent causing abnormality, since they too became diseased within a short time. The development of the disease in the normal sectors was identical to that in normal colonies whose advancing hyphae have made contact with diseased hyphae. The manner in which the normal sectors of *H. victoriae* developed from subcultures of the diseased fungus was similar to the sectors of rapid, unlimited growth of *str* (streak) mycelium that developed from the slower growing *str′* colonies of Schizophyllum reported by Papazian (5). After development, however, the *str* sectors were not affected by contact with the *str′* portion of the colony.

Comparative pathogenicity of normal and diseased H. victoriae.—1) *Leaf inoculations.*—The pathogenicity of normal and diseased *Helminthosporium victoriae* was compared by placing the inoculum at the base of the primary leaf blade of susceptible oats. The inoculum was 3-mm cubes of mycelial mat plus agar of the two growth types, covered by a moistened piece of absorbent cotton. The inoculated plants were placed in a moist chamber for 48 hours and then removed to the greenhouse bench. The experiment was conducted twice, with each treatment containing 10 pots with 10 plants per pot.

Large necrotic lesions developed at the site of inoculation with both diseased and normal *H. victoriae* within 4–5 days, and necrotic streaking of the primary leaf soon followed. Thereafter, differences in the development of the disease were marked. The crown and basal portions of plants inoculated with normal *H. victoriae* turned brown, and within 10 days such plants developed severe crown and root rot and necrotic streaking of all of the leaves. Occasionally a plant inoculated with diseased *H. victoriae* developed the severe symptoms described above and was killed. Ordinarily, however, typical Victoria blight failed to develop after the necrotic streaking and death of the primary leaf.

In the two experiments involving 200 plants for each of the two treatments, 174 plants survived inoculation with diseased *H. victoriae* (Fig. 1-B), but none survived inoculation with normal *H. victoriae* (Fig. 1-A).

2) *Inoculation of the soil.*—In 1947 Litzenberger and Murphy (3) described a method of inoculation for determining resistance of oats to *Helminthosporium victoriae*. A mycelial suspension of the fungus, with or without agar, applied to the soil with the grains gave no survival in susceptible varieties. The method was used in this study to test the relative pathogenicity of normal and diseased *H. victoriae* and to determine the extent of disease development when the two types of growth were inoculated in combination. Aliquots of 250 ml of inoculum of diseased, normal, and diseased-plus-normal *H. victoriae* in combination were mixed with 25 oat grains and added to soil in 6-in. pots. There were 10 pots in each treatment, and the average number of plants per pot after emergence was 22 in all treatments. The first symptoms of the disease appeared about 2 weeks after planting. Plants considered to have survived were those showing only slight brownish discoloration of the crown and roots and no necrotic streaking of the leaves.

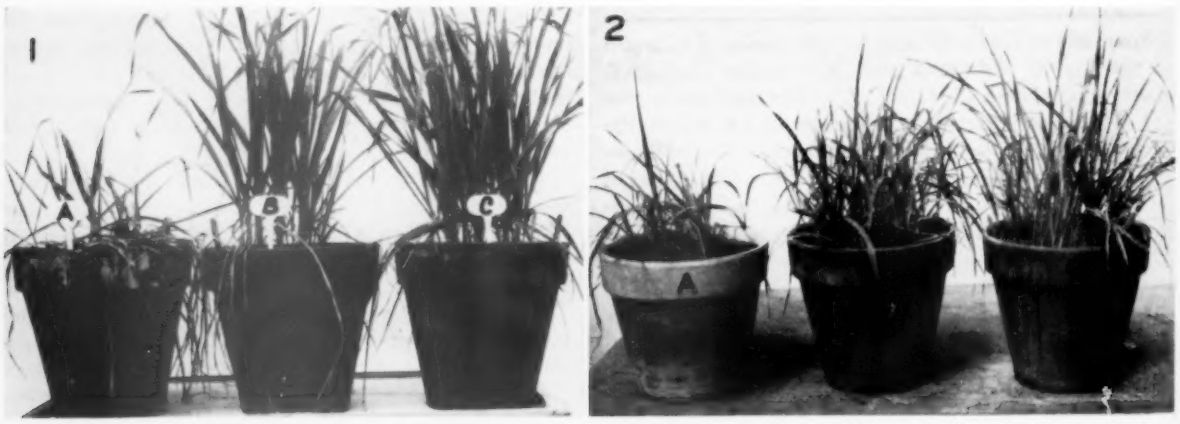


Fig. 1–2.—1) Comparative pathogenicity of A) normal *H. victoriae* and B) diseased *H. victoriae* applied by inoculation of the primary leaf. Healthy controls are at C. 2) Comparative pathogenicity of A) normal *H. victoriae*, B) normal and diseased *H. victoriae* in combination, and C) diseased *H. victoriae* added to soil with the grains of susceptible oats.

The data in experiments I and II (Table 1) show that the mean number of plants surviving inoculaton with diseased *H. victoriae* (Fig. 2-C) was significantly greater than the number surviving inoculation with the normal fungus (Fig. 2-A). The mean number of plants surviving inoculation with normal and diseased *H. victoriae* in combination (Fig. 2-B) was intermediate to the others, and significantly dfferent. With the pathogenicity of normal *H. victoriae* apparently reduced by the presence of diseased *H. victoriae*, additional investigations were carried out to detect further changes in pathogenicity.

The effect of time on the pathogenicity of the normal portion of the inoculum in combination with diseased *H. victoriae* was tested by delaying the planting of susceptible oats. The studies were similar to those of the above experiments except that the inocula were added to sterile soil in 6-in. pots 30 days before planting, or by replanting oats in the previously inoculated soils of experiments I and II 30 days after removal of their test plants. The unplanted soils were watered lightly to avoid excessive drying. Plants were evaluated similarly to those of experiments I and II.

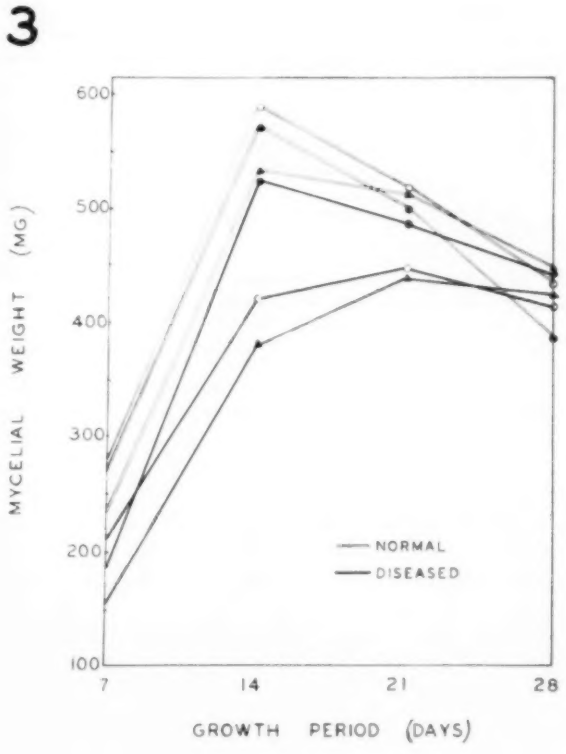
The data of experiments III, IV, V, and VI (Table 1) show that the mean number of plants surviving inoculation with normal and diseased *H. victoriae* in combination were almost equal to the average number surviving treatment with diseased fungus only. The mean number of plants surviving inoculation with normal *H. victoriae* remained relatively low, though higher than the number surviving the same treatment at the time of planting of the oats.

Table 1.—Comparative pathogenicity of diseased, normal, and diseased and normal *H. victoriae* in combination by inoculation of the soil.

	Mean number of surviving plants in experiments[a]					
	I	II	III	IV	V	VI
Normal	1.5	1.7	4.0	3.2	6.2	6.3
Diseased + Normal	9.9	12.3	15.5	14.2	18.3	17.1
Diseased	17.4	18.1	16.6	18.3	18.6	19.0
LSD 5%	1.6	1.5	1.0	1.2	1.1	1.1
1%	2.2	2.1	1.4	1.6	1.5	1.5

[a] I, II: inoculum added to soil with the grains of susceptible oats; III, IV: soil from Expt. I and II and reseeded with susceptible oats after 30 days; V, VI: soil infested with the fungus 30 days before being seeded with susceptible oats.

Comparative growth and toxin production of normal



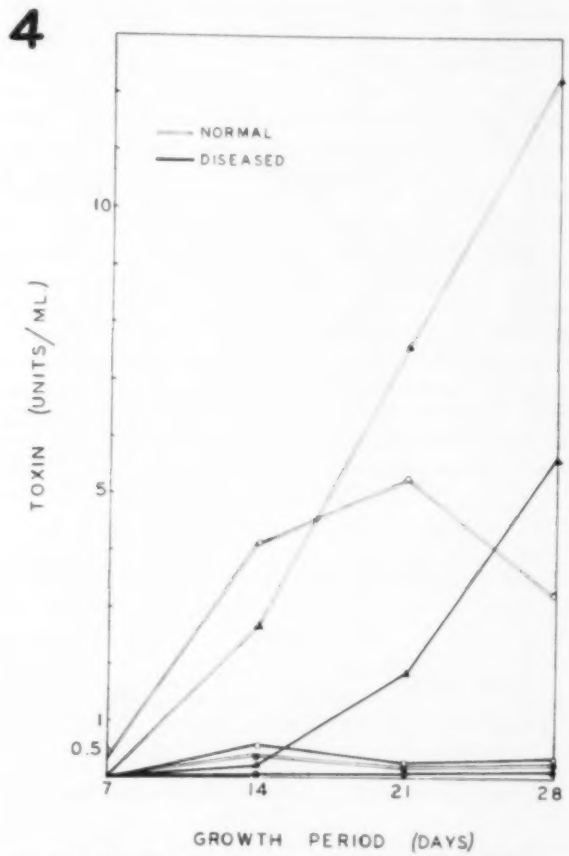


Fig. 3–4.—3) Growth of normal and diseased *H. victoriae*, respectively represented by parallel lines and solid lines. Solid triangles, 24°C; open circles, 28°C; and solid circles, 32°C. 4) Toxin production by normal and diseased *H. victoriae*, respectively represented by parallel and solid lines. Solid triangles, 24°C; open circles, 28°C; and solid circles 32°C.

and diseased H. victoriae.—Luke and Wheeler (4) reported a correlation between growth and the production of a toxin (victorin) in pathogenic isolates of *H. victoriae.* Bioasssays indicated lower toxin production in slower-growing pathogenic isolates of *H. victoriae* than in vigorously growing pathogenic isolates.

Among the effects of the transmissible disease on *H. victoriae* was growth markedly slower than in normal fungus. The growth of diseased *H. victoriae* was inhibited to a much gretater extent on solid media than on liquid media. This was undoubtedly partly due to the fact that on liquid media the conidia were easily separated from the diseased growth and produced normal and independent growth for varying periods. Nevertheless, a study was made of growth and toxin production on a liquid medium to determine whether any reduction in diseased growth was accompanied by a corresponding reduction in toxin production.

Diseased and normal *H. victoriae* were seeded in 50 ml of a modified Fries' medium in 500-ml Erlenmeyer flasks and incubated at 24, 28, and 32°C. Growth and toxin determinations were made at 7, 14, 21, and 28 days after seeding. The mycelial mats of normal and diseased *H. victoriae* from 5 flasks of each type of growth and at each of the three temperatures were harvested, dried, and weighed. The samples for toxin determinations were taken from a mixture of the media of the 5 flasks. Bioassay of the toxin and the method for determining units of toxin per ml have been described by Luke and Wheeler (4).

Irrespective of growth (Fig. 3), toxin production (Fig. 4) of both normal and diseased *H. victoriae* at 32°C was very low, and is hereinafter disregarded. The data in Fig. 3 show that at 24 and 28°C the growth of normal *H. victoriae* was greater than that of the diseased fungus, but that after 14 days the drop in weight of the normal growth was more rapid than that of the diseased growth.

The results in Fig. 4 show that at temperatures favorable to toxin production (24 and 28°C) toxin activity was 2–10 times as great in medium supporting normal *H. victoriae* as in medium from diseased cultures. Toxin activity in the early stages of growth (7–14 days) was greater at 28°C, but thereafter was favored by and reached a maximum at 24°C. Thus the reduced growth of diseased *H. victoriae* resulted in toxin production that was lower than that of the more rapidly growing, normal *H. victoriae.* The pattern is similar to that found in the pathogenic isolates studied by Luke and Wheeler (4).

Discussion.—There is no proof that the abnormality in *H. victoriae* has been effective in reducing the pathogenicity of the fungus in nature. In recent years, however, there have been fewer epiphytotics of Victoria blight in the South, even though considerable (though less extensive) acreages of oats with Victoria parentage have been planted. Ivanoff et al. (1) reported that Victoria blight in south Mississippi in the 1956–57 season was the first large-scale appearance of the disease since the epiphytotic of 1946 to 1948. In November of 1958, extensive leaf spotting incited by *H. victoriae* was observed in south Louisiana fields of Victorgrain 48-93. Both normal and diseased *H. victoriae* were isolated from these oats at that time. Despite the disease, the fields yielded good grazing and the stands were almost undiminished. Such a pattern of disease development was not regarded as typical for oats infected with *H. victoriae.*

The possibility of natural or biological control of a disease by such a phenomenon as a transmissible disease of the pathogen involved is only suggested. Further work is in progress on this interesting problem.—Department of Plant Pathology, Louisiana Agricultural Experiment Station, Baton Rouge.

LITERATURE CITED

1. Ivanoff, S. S., D. H. Bowman, and P. G. Rothman. 1958. Oat diseases in Mississippi. Plant Disease Reptr. 42:521-523.

2. Lindberg, G. D. 1959. A transmissible disease of Helminthosporium victoriae. Phytopathology 49:29-32.

3. Litzenberger, S. C., and H. C. Murphy. 1947. Methods for determining resistance of oats to Helminthosporium victoriae. Phytopathology 37:790-800.

4. Luke, H. H., and H. E. Wheeler. 1955. Toxin production by Helminthosporium victoriae. Phytopathology 45:453-458.

5. Papazian, H. P. 1955. Sectoring variants in Schizophyllum. Am. J. Botany 42:394-400.

POTATO VIRUS F LATENT IN AN IMPORTED VARIETY AND RESISTANCE TO THE VIRUS IN AN INTERSPECIFIC POTATO HYBRID

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SUMMARY

A virus recovered from outwardly healthy plants of the Dutch potato variety Albion, and one recovered from a potato seedling, were shown to conform to descriptions of potato virus F. An interspecific potato hybrid (*Solanum tuberosum* var. Epicure × 4n *S. chacoense*) was found to be highly resistant to virus F.

Potato virus F (7), which is considered to include the related "virus G," was found some years ago in older potato varieties grown on a small scale in Eastern Canada (11, 15), but the virus has never been reported from commercial seed-growing areas of Canada or the United States. This is despite its occurrence in potatoes throughout Europe (5, 6, 7, 12, 14, 17, 18, 19, 20, 21) as well as in South America (8), Africa (9), and Australia (4), and even in wild *Solanum* species from Peru (10). Virus F has been

associated with an "interveinal mosaic" disease (18), with a "streak" (11), and with "tuber blotch" (6, 14). "Virus G" is the incitant of the "aucuba mosaic" disease (7, 17). Virus F appears forbidding in some of its symptoms, but is sporadic and usually of minor importance.

My interest was drawn to virus F indirectly in 1953. Some scions of Irish Cobbler potato used to infect other varieties with potato virus X developed necrotic lesions 2 weeks after being grafted to plants of the Dutch variety Albion. The Albion was outwardly healthy and had long been grown as such in American and Canadian variety collections. This suggested the presence of a virus latent in the Albion, and it was subsequently recovered and inoculated to a series of differential host plants. These tests indicated that the virus was related to virus F, for it systemically infected virus-X-immune U.S.D.A. Potato Seedling 41956 and virus-Y-immune *Datura tatula* L., and incited distinct local lesions and systemic necrosis (Fig. 1-A) in *Capsicum annum* L. (3). In 1956, a similar virus was recovered from a severely necrotic plant of Potato Seedling F451, found in a greenhouse at the Fredericton Research Station by D. F. Rankin.

To confirm that the viruses from Albion and Seedling F451 were in fact virus F isolates, they were compared with other known strains, including type strains of virus F and "virus G" from Holland, obtained in 1955 from Rozendaal (20) through the courtesy of R. H. Larson, University of Wisconsin, and Clinch, Loughnane and Murphy's (7) type strain of virus F from Ireland, supplied by Munro (16) in 1958. Comparison was also made, more recently, with an isolate of virus F that Munro found in Southesk potato.

Antiserum against the virus from Albion reacted equally well with that virus, the one from Seedling F451, the three type strains of virus F, and the Southesk virus. The titer of the strongest antiserum was within the range 1/512–1/1024 when tested against each of the six virus F isolates. The first antiserum was prepared by injecting a rabbit with sap from infected tobacco, but the antiserum would not give a visible precipitate with clarified sap from infected tobacco or potato. It did so, however, with sap from infected—though not from healthy—*C. annum* and *D. tatula*. These two species thus seem to be better sources than either tobacco or potato.

The serological tests left little doubt that the two viruses I had found were isolates of virus F. Significant, too, is the evidence that the relationship between "virus G" and the type strains of "virus F" is of the order usually ascribed to strains of the same virus. This confirms a suggestion by Dykstra (11) that the viruses of "aucuba mosaic" and "tuber blotch" are related, although the relationship is closer than he appeared to realize. The name virus F is therefore considered to apply to all six of the isolates mentioned here.

Subsequent tests of other stocks of Albion received from the Inter-regional Potato Introduction Station, Sturgeon Bay, Wisconsin, and from L. C. Young, Fredericton Research Station, showed that these invariably contained virus F. Since all of these Albion were derived from tubers imported by the U.S.D.A. in 1934 (22), it is probable that the imported tubers carried the virus.

Host-range studies.—*Differential hosts.*—These encounters with virus F in experimental potato stocks where its presence had not been suspected led to a brief further study of the virus to aid in its possible future diagnosis. Of 31 Solanaceae inoculated with sap containing the Albion isolate of virus F, all but one—*Solanum capsicastrum* Link. ex. Schau.—became infected. Many of these species were symptomless, but whitish specks or blotches appeared systemically on the leaves of *Datura tatula* (Fig. 1-B) and a number of other species. Further tests, however, showed that these symptoms were either not typical of all isolates of virus F or did not consistently appear on infected plants. None of the species tested was a more reliable indicator than *C. annuum*, which reacted with necrotic local lesions and systemic necrosis to all of the virus F isolates (Fig. 1-A). This was true under ordinary greenhouse conditions prevailing throughout the year as well as in a greenhouse with temperature controlled at 18–20°C. The necrotic lesions first appeared in 4–10 days as light gray, etchlike, concentric-ring lesions on the upper surface or as dark gray, sunken, circular spots, 3 mm in diameter, on inoculated primary leaves. Some leaves had both types of lesion. The etchlike lesions were often faint and isolated but were sometimes quite distinct and also coalesced, as illustrated. The necrotic tissue of the sunken lesions dried white, surrounded by a brownish halo, if the leaves remained on the plant long enough. Systemic lesions, either elongate near the veins, or ringlike in the interveinal areas, appeared on successively higher leaves, being gray or white on the middle leaves, but more pronouncedly brown on the upper leaves of each shoot. Leaves with necrotic lesions eventually dropped. Young *C. annuum* plants were sometimes killed in a week, but older plants lingered for months, defoliated except for a few apical or axillary buds.

Solanum miniatum Bernh., recently described as an indicator host for virus F (16), was found useful for this purpose. But its reaction to the Albion isolate under all conditions and to the other isolates at temperatures below 20°C was comparatively mild.

Neither *C. annuum* nor *S. miniatum* could be depended upon for diagnosis of virus F if virus X was also present. Practically every symptom incited by virus F was duplicated or obscured by one strain or another of virus X.

Localized infections with virus F also occurred in leaves of two species of Amaranthaceae—symptomlessly in *Gomphrena globosa* L. and inciting brown necrotic lesions in *Amaranthus retroflexus* L. One species of Leguminosae, *Cyamopsis tetragonaloba* Taub., developed on inoculated cotyledons small ne-

crotic lesions similar to those incited by potato viruses S and M (3).

Virus F in potatoes.—Plants of some North American commercial varieties were inoculated with the Albion isolate of virus F and held for observation in a greenhouse where temperatures were usually within the range of 20–24°C. The virus incited necrotic local lesions, often followed by severe systemic foliar necrosis, when introduced by sap inoculation into the varieties Bliss Triumph, Canso, Chippewa, Irish Cobbler, Katahdin, Kennebec, Russet Burbank, Saco, Sebago, and Teton. These varieties reacted with top necrosis when virus F (Albion) was introduced by grafting. Internal necrotic lesions frequently resulted from sap or graft inoculation in tubers of these varieties. Secondary plants from such tubers, if they

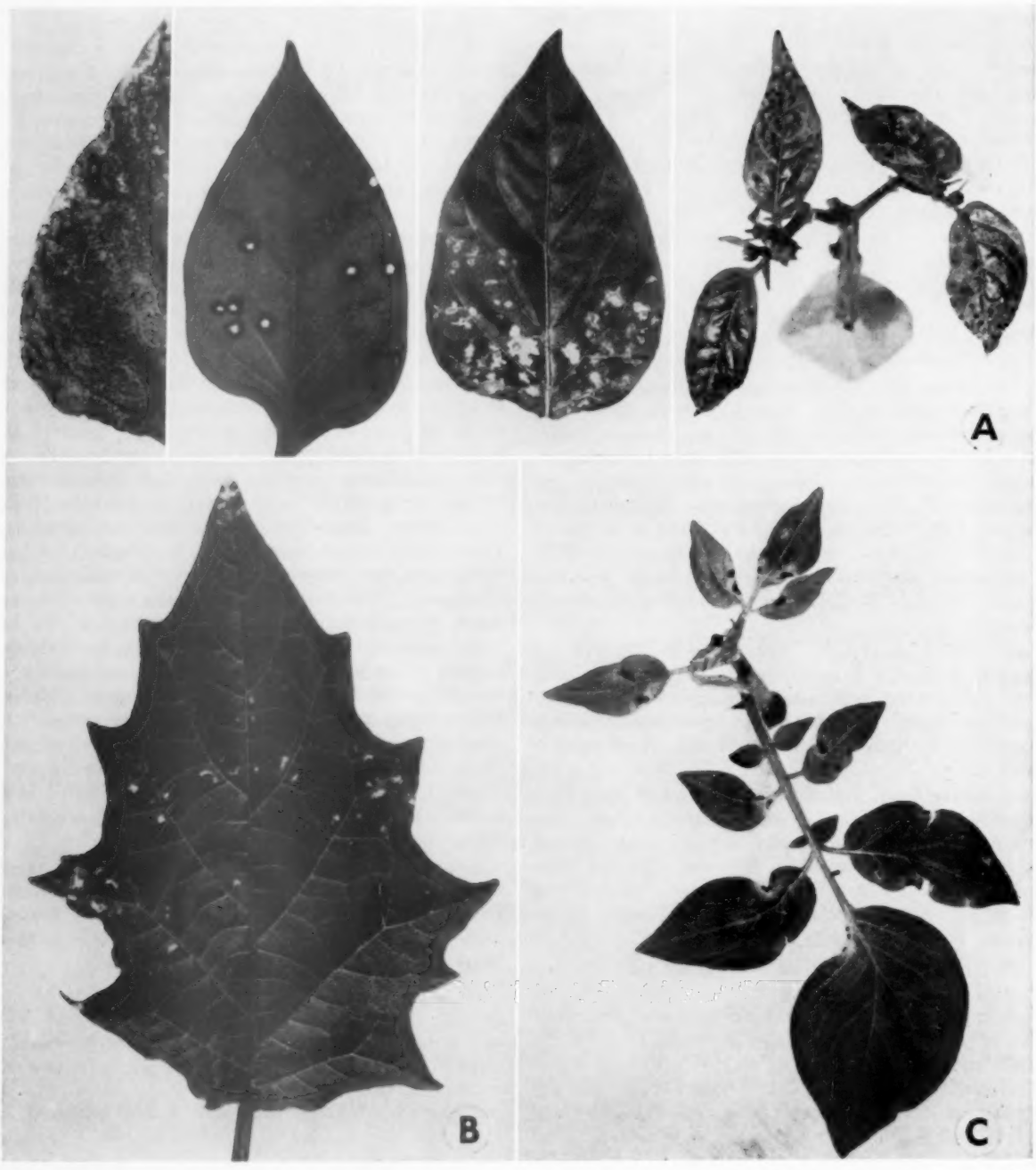

Fig. 1.—Symptoms incited by virus F (Albion isolate). **A)** *Capsicum annuum*. L–R: "Etch-like" local lesions, "sunken necrotic" local lesions, systemic lesions on a middle leaf, and systemic lesions on upper leaves. **B)** Whitish necrotic specks appearing systemically on *Datura tatula*. **C)** Systemic necrotic lesions on a P.I. 197772 shoot following graft inoculation with infected scion.

emerged at all, were usually stunted and showed varying degrees of foliar necrosis. Tubers from several necrotic Saco plants, however, gave rise to healthy progeny, which suggests that the necrotic reaction has some bearing upon varietal resistance. The variety Green Mountain and U.S.D.A. Seedling 41956 appeared more tolerant of virus F (Albion) than did the necrotic-reacting varieties just mentioned. They sometimes developed a mild foliar necrosis when first inoculated, but plants grown from infected tubers of Green Mountain developed only a faint interveinal mosaic, and secondary plants of Seedling 41956 were symptomless.

In further trials Seedling 41956 and the variety Saco were inoculated with sap containing each of the 6 different isolates of virus F. Five of the isolates infected Seedling 41956 without causing symptoms. But foliage of plants infected with the "virus G" isolate developed small conspicuous yellow spots, first on the lower leaves and later on successively higher leaves, reminiscent of "aucuba mosaic." Saco plants reacted to 3 of the virus F isolates, with necrotic local lesions and severe foliar necrosis, but carried the "Southesk," "aucuba" and "Irish-F" isolates symptomlessly.

A number of wild tuber-bearing *Solanum* species and interspecific hybrids were also inoculated with virus F (Albion). Plants of U.S.D.A. Plant Introduction 197772 (*Solanum tuberosum* L. var. Epicure × 4n *S. chacoense Britt.* (13)) remained free of symptoms, whereas several other species and hybrids developed necrotic symptoms. Despite repeated efforts to infect P.I. 197772 by sap inoculation with each of the six isolates of virus F, the virus was never recovered from foliage of the inoculated plants by return inoculation to *C. annuum*. In the meantime, plants of P.I. 197772 were infected with viruses M, S, and X when each of these viruses was applied simultaneously with virus F in sap inoculum. P.I. 197772 is also susceptible to virus Y but is hypersensitive to virus A.

Plants of P.I. 197772 developed a form of foliar or "top" necrosis (Fig. 1-C) when each of the six isolates of virus F was introduced by top-grafting with infective scions. A few scattered lesions appeared on the uppermost leaves of shoots growing from leaf axils below the graft 10–16 days after grafting. Apical growth of the P.I. 197772 shoots was little affected, and lesions appeared on each new leaf. The lesions developed slowly and eventually appeared to be localized. Virus F could be recovered on *C. annuum* test plants sometimes from leaves showing fresh lesions but not from older leaves. Traces of necrosis also appeared in one tuber of a P.I. 197772 plant infected by graft inoculation with virus F (Albion). This necrotic tuber and other tubers saved from 18 of the necrotic-reacting P.I. 197772 plants produced healthy-appearing plants from which virus F could not be recovered on *C. annuum*.

A selection of 2n *S. chacoense* (P.I. 175, 443 (13)) was also inoculated with virus F (Albion), but the virus was not recovered. Further, all of 24 tested seedlings of P.I. 197772, selfed, appeared to be resistant to virus F.

Discussion.—The presence of latent viruses in potatoes kept for experiment and breeding seems to have received little attention. Viruses S and X are, of course, common in susceptible varieties, but in addition to virus F in Albion, I have found virus A in Golden Wonder, virus M in Fortuna, King Edward and Saco, and virus Y (c-strain) in Thorbecke. All of these varieties were symptomless and, moreover, disarmingly "free of virus X." Dykstra's (11) suggestion that American potato pathologists be alert for tuber necrosis, typical of virus F, has brought no response in 20 years. Virus F exists in a number of strains inciting a wide range of symptoms—often erratic in appearance—in potatoes and other hosts. It is possible that an alert for latent viruses would be more successful.

The plants of seedling F451, from which virus F was isolated, were growing among healthy plants of this seedling in a greenhouse planting that included some 30 different varieties and seedlings. The Albion variety was not present but it is possible that some other variety carries virus F. The Albion and F451 isolates do not appear to be identical. Little is known about the spread of virus F, though Quanjer, Thung, and Elze (19), and Clinch, Loughnane, and Murphy (7) found that this virus was transmitted by aphids.

The resistance of P.I. 197772 to virus F appears considerably greater than that of any commercial potato variety tested. Necrotic response, often termed "intolerance" or "hypersensitivity," has been correlated with field resistance to potato viruses A, X, and Y, the reaction being specific toward the individual virus. The effectiveness of this type of resistance varies. I have found strong resistance to virus A in intolerant potato varieties (1), but resistance moderate by comparison in varieties that react necrotically to virus Y (2). Typical of the stronger resistance to virus A is a practical immunity to the virus in sap inoculum and slow development of top necrosis once infection is initiated by grafting. The most intolerant of the commercial potato varieties tested were readily infected by sap inoculation with virus F and succumbed rapidly when infected by grafting. But plants of P.I. 197772 appeared to be immune to virus F in sap inoculum and showed an ability to localize the necrotic lesions when infected by grafting.—Canada Department of Agriculture, Research Station, Fredericton, New Brunswick.

LITERATURE CITED

1. Bagnall, R. H. 1953. Notes on potato virus A. (Abstr.) Can. Phytopathol. Soc. Proc. 20:11.

2. Bagnall, R. H., and R. H. E. Bradley. 1958. Resistance to virus Y in the potato. Phytopathology 48:121-125.

3. Bagnall, R. H., R. H. Larson, and J. C. Walker. 1956. Potato viruses M, S and X in relaton to interveinal mosaic of the Irish Cobbler variety. Wisconsin Univ. Agr. Expt. Sta. Research Bull. 198, 45 p.

4. Bald, J. G. 1937. An F-type potato virus in Australia.

Nature, (London) 139:674.

5. Bjornstad, A. 1948. Virussjukdommer pa Potet i Norge. Nord. Jordbrugsforsk., 1948:586-590. (Abstr. in Rev. Appl. Mycol. 29:635).

6. Clinch, Phyllis E. M. 1941. A strain of the tuber blotch virus causing top necrosis in the potato. Sci. Proc. Roy. Dublin Soc. (NS) 22:435-445.

7. Clinch, Phyllis E. M., J. B. Loughnane, and P. A. Murphy. 1936. A study of the aucuba or yellow mosaic of the potato. Sci. Proc. Roy. Dublin Soc. (NS) 21:431-448.

8. Costa, A. S., and H. P. Krug. 1937. Molestias da Batatinha em São Paulo. Bol. Inst. agron. Campinas 14, 55 p. (Abstr. in Rev. Appl. Mycol. 17:57.)

9. De Carvalho, T. 1948. Relação preliminar de doenças encontradas em plantes e insectos com anataçoes fitapathológicas. (Mozambique) Reparticae de Agricultura, Seccao de Micologia, 84 p. (Abstr. in Rev. Appl. Mycol. 29:89.)

10. Dennis, R. W. G. 1939. Notes on the photoperiodic reactions and virus contents of some Peruvian potatoes. Ann. Appl. Biol. 26:87-101.

11. Dykstra, T. P. 1939. A study of viruses causing yellow mosaics in European and American varieties of the potato, Solanum tuberosum. Phytopathology 29:917-933.

12. Gulyoa, A. 1938. A Burgonya virusbetegségei. A virusok jelentögsége a leromlásban és az ellenük való védekezések. M. Kir. Gazdaz. Akad. Mun. (Hungary) I, 3, 63 p. (Abstr. in Rev. Appl. Mycol. 17:619).

13. Hougas, R. W. (Project Leader). 1958. Inventory of tuber-bearing solanum species. Wisconsin Univ. Agr. Expt. Sta. Bull. 533, 40 p.

14. Loughnane, J. B., and Phyllis E. M. Clinch. 1935. Composition of interveinal mosaic of potatoes. Nature, (London) 135:833.

15. MacLeod, D. J. 1944. The mosaic types of viruses affecting potatoes in Canada and Great Britain. Ph. D. thesis, Cambridge University, England.

16. Munro, J. 1959. Diagnosis of potato virus F by means of Solanum miniatum. Can. J. Botany 37:903-905.

17. Quanjer, H. M. 1921. New work on the leaf curl and allied diseases in Holland. Roy. Hort. Soc., London, Rept. Intern. Potato Conf. Rept. p. 127-145.

18. Quanjer, H. M. 1923. General remarks on potato diseases of the curl type. Intern. Conf. Phytopathol. and Econ. Entomol., Holland, Rept. p. 23-28.

19. Quanjer, H. M., T. H. Thung, and D. L. Elze. 'Pseudonetnecrose' van de Aardappel. (Wageningen) Landbouwhoogesch. Meded. 33, 9, 10 p. (Abstr. in Rev. Appl. Mycol. 9:483).

20. Rozendaal, A. 1952. Demonstration of experiments with potato viruses. Proc. Conf. Potato Virus Diseases, Wageningen-Lisse, 1951, p. 63-65.

21. Sardina, J. B. 1945. Enfermedades de la Patata. Publ. Estac. Fitopat. Agríc. Coruña 5, 111 p. (Abstr. in Rev. Appl. Mycol. 25:228.)

22. Stevenson, F. J. 1948. Foreign potato introductions. Am. Potato J. 25:259-265.

A NECROTIC POD STREAK OF PEPPER CAUSED BY TOBACCO MOSAIC VIRUS

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Journal Paper No. 2552 of the Michigan Agricultural Experiment Station. The writer thanks Dr. F. O. Holmes for the culture of a distorting strain of TMV, seeds of Samsun tobacco, and LL-resistant garden pepper; and Dr. W. N. Takahashi for seeds of Xanthi tobacco.

SUMMARY

Necrotic pod streak, previously unreported in pepper (*Capsicum annum* L.) was reproduced experimentally in parallel inoculation tests with virus isolated from diseased pods and with Holmes' distorting strain of tobacco mosaic virus (TMV). Plants inoculated at first flowering usually expressed necrotic pod streak and stem necrosis in about 4–6 weeks, but inoculation of plants bearing immature pods resulted in an internal pod necrosis. Pod streak symptoms occurred at 28, 22, and 17°C, but only the small-fruited Hungarian Sweet and 2 other related varieties were susceptible. None of the large-fruited types tested developed pod necrosis. The pepper virus, shown to be a strain of TMV, differed from Holmes' distorting strain in its failure to incite local lesions on Pinto and Scotia bean and from Miller and Thornberry's tomato atypical mosaic virus in thermal inactivation, 93°C for 10 minutes.

A previously undescribed disorder of a small-fruited variety of pepper (*Capsicum annum* L.) caused growers in southeastern Michigan an estimated 20–30% loss in 1956, and again in 1957. The disease was observed only on Hungarian Sweet pepper, a processing variety intensively grown in this area. The disorder, which rendered pods unacceptable to processors, was characterized by slightly raised, reddish-brown streaks about 5–50 mm long and 1–8 mm wide on the fruit surface (Fig. 1-A). Plants bearing streaked pods appeared partially defoliated, and sectioned stems of such plants showed necrosis in portions of the pith (Fig. 1-C). Various pod disorders (1, 2, 4, 7, 8, 10, 11) and stem necrosis of pepper (3, 5, 6) have been described, but no reference to a similar necrotic streak of the pod was found in the literature.

Materials and methods.—Inoculations were usually made by homogenizing leaf, stem, or pod tissues in 1% K_2HPO_4 buffer to give a 1:50 or 1:100 dilution. The juice was applied with a cotton swab on host plants previously dusted with fine carborundum powder. Except in the controlled temperature studies, all inoculations were made in the greenhouse at 20–30°C. Seeds of host plants were from commercial sources unless stated otherwise. All test plants were grown in 4-in. pots containing a steamed soil-sand-peat (3:1:1) mixture.

Results.—Microscopic examination and culturing of necrotic fruit tissue revealed no bacteria or fungi. Portions of diseased pods were then triturated in phosphate buffer and the juice used to inoculate plants of *Nicotiana tabacum* vars. Samsun and Xanthi, *C. frutescens* L. var. Tabasco, and *Phaseolus vulgaris* L. vars. Pinto and Scotia. Local lesions in Samsun were followed by systemic mottle, whereas Tabasco and Xanthi leaves developed local lesions only. The 2 bean varieties remained symptomless. The virus isolated in Samsun tobacco was maintained in this host and used in further tests.

Experimental reproduction of pod symptoms—At-

tempts were made to reproduce the necrotic pod-streak symptoms by inoculation with the virus to Hungarian Sweet pepper plants at different stages of maturity. One group was 5 plants at first flowering; the second group was 5 plants with immature pods of ⅛–¼ size, and the third group was 2 plants with nearly mature pods. Comparable numbers of plants in these stages were placed on the same bench as controls. Within 3–5 days, all inoculated leaves developed local chlorotic and necrotic spots. Leaf casting and blossom drop followed in 2–3 more days. In the second group of plants the inoculated pods developed a grayish-brown translucent internal necrosis (Fig. 1-B) and dropped prematurely. In the third group, the internal pod-necrosis system was only slightly perceptible. In neither of these 2 groups with pods did the characteristic raised, necrotic streaks appear at this time but characteristic symptoms appeared on pods that set later on these plants. Plants in the first group, flowering when infected, later set pods. On these, original pod symptoms appeared 6 weeks from the time the leaves were rubbed. The necrotic pith areas in the stems of inoculated plants were like those observed in the field. Reisolation from the necrotic tissues yielded local lesions on Xanthi tobacco.

Effect of temperature on pod symptom development.—Samuel (9) showed that *N. glutinosa* plants inoculated with TMV (tobacco mosaic virus) responded at high temperature with systemic symptoms rather than the normal local lesions. To determine whether the stem and pod symptoms in Hungarian Sweet pepper might be the result of a similar temperature-host interaction, a test was made under controlled temperatures in the greenhouse. Groups of 12 Hungarian Sweet pepper plants at first flowering were inoculated with the pepper virus and placed at 28, 22, and 17°C. Similar groups of 12 plants each were inoculated with Holmes' distorting strain of TMV and kept at the same temperatures. Among plants inoculated with the pepper virus, necrotic pod streaks developed in 4 at 28°C in 26 days, in 4 at 22°C in 32–34 days, and in 7 at 17°C in 39–57 days. Among plants inoculated with TMV, all at 28°C exhibited stunting and severe blossom drop resulting in complete unfruitfulness, 4 at 22°C had pod necrosis at 32–34 days, and 4 at 17°C had pod necrosis at 27–59 days. At each temperature level, all plants with necrotically streaked pods also showed stem necrosis.

Varietal reaction.—When large-fruited mild pepper types were inoculated, both the pepper isolate and TMV induced similar symptoms. The main reactions of the varieties tested were: local lesions only (Yolo Wonder and Holmes' LL resistant garden pepper); local vein necrosis with little or no systemic spread (Allbig); mottling followed by light to moderate necrotic, stem lesions (California Wonder and Pimiento Perfection); mottling followed by severe stem lesions or lethal necrosis (New Jersey 13, Penn Wonder and Sunnybrook). None of these varieties reacted with pod-streak symptoms.

Small-fruited pungent and mild pepper types and Pinto and Scotia bean were tested in parallel inoculations. The results indicate that necrotic pod-streak symptoms were induced in Hungarian Yellow Wax, Hungarian Sweet, and Yellow Long Sweet. The pungent varieties Serrano, Red Chili, and Long Red Cayenne were without noticeable pod symptoms. Tabasco responded with local lesions to TMV and to the pepper virus, whereas Long Red Cayenne developed systemic mottle and stem necrosis. In the last host, TMV induced a more pronounced yellow mottle than did the pepper isolate. Lethal necrosis was observed in certain plants of Red Chili and Serrano, but even those that survived produced normal-appearing pods. Although the pepper varieties responded very similarly to both the pepper virus and TMV, Pinto and Scotia

Fig. 1.—**A)** Raised necrotic streaks on pods of Hungarian Sweet Pepper, induced by TMV inoculation. **B)** Internal pod necrosis. **C)** Stem necrosis affecting pith. Left, infected stems; right, healthy.

bean reacted differently. TMV caused local lesions on both bean varieties, but, again, both failed to respond to the pepper isolate.

Thermal inactivation of pepper virus.—Miller and Thornberry (6) reported a tomato atypical mosaic virus (TAMV) that, though identical to common TMV in symptoms induced in pepper varieties, differed from TMV in thermal stability and in not being able to incite local lesions on 5 varieties of bean, including Pinto and Scotia. Pod symptoms of inoculated plants apparently were not studied, but both TAMV and TMV caused similar necrotic stem lesions in 7 pepper varieties. TAMV was differentiated from TMV on the basis of noninfectivity to bean and on the low thermal inactivation of their virus, 72°C for 10 minutes. Because of similarities to TAMV in host range and symptomatology, the thermal inactivation point of the pepper pod-necrosis virus was determined. Three 2-ml samples of crude sap, expressed from frozen leaves of a Samsun tobacco plant systemically infected with the pepper virus, were respectively heated for 10 minutes in small thin-walled tubes in a water bath at 85, 90, and 93°C. The tubes were then cooled and the juices rubbed on leaves of healthy Samsun plants. Local lesions followed by systemic mottle developed on plants inoculated with juices heated at 85 and 90°C, but leaves rubbed with juice heated at 93°C remained symptomless. Subinoculations from the systemically infected Samsun plants to 2 groups of Hungarian Sweet plants reproduced the original pod and stem symptoms. The high thermal inactivation of the pepper pod-necrosis virus is characteristic of TMV and differentiates the virus from Miller and Thornberry's TAMV.

Conclusion.—The pepper pod-necrosis virus in question is shown to be a strain of TMV that produces no symptoms on bean. The studies also indicate that necrotic pod streak of Hungarian Sweet pepper resulting from TMV infection is probably a response associated with this variety or related varieties. Though greenhouse tests showed that pod symptoms were expressed over a wide temperature range, the disorder probably was not noted earlier because Hungarian Sweet pepper may be less widely grown than the large-fruited types, which did not react with necrotic pod symptoms.—Department of Botany and Plant Pathology, Michigan State University, East Lansing.

LITERATURE CITED

1. Anderson, C. W., and M. K. Corbett. 1957. Virus diseases of peppers in central Florida survey results 1955. Plant Disease Reptr. 41:143-147.

2. Doolittle, S. P., and W. J. Zaumeyer. 1953. A pepper ringspot caused by strains of cucumber mosaic virus from pepper and alfalfa. Phytopathology 43:333-337.

3. Greenleaf, W. H. 1953. Effects of tobacco-etch virus on peppers (Capsicum sp.). Phytopathology 43:564-570.

4. Higgins, B. B. 1922. The bacterial spot of pepper. Phytopathology 12:501-516.

5. Holmes, F. O. 1937. Inheritance of resistance to tobacco-mosaic disease in pepper. Phytopathology 27:637-642.

6. Miller, P. M., and H. H. Thornberry. 1958. A new viral disease of tomato and pepper. Phytopathology 48: 665-670.

7. Ramsey, G. B., J. S. Wiant, and L. P. McColloch. 1952. Market diseases of tomatoes, peppers, and eggplants. U. S. Dept. Agr. Handbook No. 28, 54 p.

8. Roque, A., and J. Adsuar. 1941. Studies on the mosaic of peppers (Capsicum frutescens) in Puerto Rico. Puerto Rico Univ. J. Agr. 25:40-50.

9. Samuel, G. 1931. Some experiments on inoculating methods with plant viruses, and on local lesions. Ann. Appl. Biol. 18:494-507.

10. Simons, J. N. 1956. The pepper veinbanding mosaic virus in the Everglades area of south Florida. Phytopathology 46:53-57.

11. Simons, J. N. 1957. Three strains of cucumber mosaic virus affecting bell pepper in the Everglades area of south Florida. Phytopathology 47:145-150.

STUDIES ON YELLOW BUD MOSAIC VIRUS

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SUMMARY

Yellow bud mosaic virus (YBMV) was mechanically transmitted to some members of the following plant families: Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Geraniaceae, Leguminosae, Malvaceae, Solanaceae, and Rosaceae. No symptoms appeared in, nor could the virus be recovered from, inoculated plant species in the Convolvulaceae, Cruciferae, Gramineae, Umbelliferae, and Vitaceae. Most useful for diagnosis were 5 herbaceous hosts: Bountiful bean, cowpea, cucumber, *Nicotiana rustica*, and *N. tabacum*. Four strains of YBMV from different hosts were classified on the diagnostic host range in the following descending order of severity: raspberry, peach, Himalaya blackberry, and Malva. The Himalaya blackberry strain, budded to peach, interfered with movement of the peach strain. Recovery of YBMV from the roots of *Malva parviflora* under an infected peach tree is believed to be its first recovery from a herbaceous plant in the field. Soil transmission of the virus appeared to be through plant roots. No transmission occurred through steamed soil. No seed transmission was demonstrated in herbaceous hosts.

Introduction.—Yellow bud mosaic is a very important virus disease of peach in some parts of California. The known host range includes many herbaceous plant species, including some important economic crops (4, 14). As the known herbaceous host range was expanded, the suggestion was made that weeds might serve as a reservoir for the virus or as an alternate host for the vector. A herbaceous host-range study was therefore undertaken. To determine the presence of

strains of YBMV a severe and a mild isolate were used in cross-inoculation tests on peach.

Literature review.—Yellow bud mosaic of peach may also damage almond trees so that they become unproductive (5). Apricot (10) and Wickson plum (11) have been naturally infected but are damaged very little as far as is known.

YBMV, also known as "Winters disease" and "Winters peach mosaic," was first found near Winters, California, in about 1936 (9). Surveys showed it to be present in both Yolo and Solano counties. Since then it has been found in San Bernardino, Placer, El Dorado, Nevada, Yuba, Napa, Butte, Sutter, and Tehama counties (7). It has not yet been reported from outside California.

YBMV differs from most other known fruit tree viruses in that infected trees are not scattered at random in the orchard but are in compact groups (8) indicating that transfer is usually from tree to adjacent tree. Occasional foci of infection are found at considerable distances from any apparent source, but spread from these foci is also to adjacent trees. In 1953, it was confirmed that healthy nursery-grown peach trees on land previously occupied by YBMV-infected peach trees became infected (11). It was also reported (12) that 9 of 22 seedlings produced by Muir peach pits in soil from around the roots of YBMV-infected peach trees developed leaf symptoms the year after being planted. Recent work (3) suggests that the soil vector of YBMV is a nematode.

YBMV was first mechanically transmitted to herbaceous hosts in 1954 (14), by a quick tissue inoculation method (13). Successful transmissions were to cowpea, tobacco, bean, sunflower, cucumber, and guar. The herbaceous host range was extended (4) to include some members of the following plant families: Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Geraniaceae, Leguminosae, Malvaceae, and Solanaceae. No symptoms appeared in, nor could the virus be recovered from, inoculated plants in the Convolvulaceae, Cruciferae, and Umbelliferae. Yarwood (15, 16) reported successful transmissions of YBMV from herbaceous plants back to peach seedlings.

It has been known for some time that some isolates of YBMV taken from different peach trees, almonds, etc., differ in severity of symptom expression on the hosts inoculated. Using concurrent inoculations of a severe and a less severe isolate of YBMV, in a vertical line on peach, H. E. Thomas (unpublished data) could demonstrate no protection by one isolate against the other. Earlier attempts (9) failed to protect peach trees against YBMV by prior inoculation with cherry rugose mosaic, plum mosaic (Santa Rosa, Vacaville), standard prune constricting mosaic, or almond calico.

In 1955, Alcorn et al (1) reported a mosaic from Himalaya blackberry that was graft-transmissible to red raspberry and peach. The symptoms produced in peach indicated a definite relation to YBMV. With inoculum prepared from cowpea, Yarwood (16) was able to transmit the blackberry virus to 1 of 25 inoculated peach trees. Most inoculations with the virus from various sources to different hosts were unsuccessful, but symptoms on the occasionally infected cowpea and peach indicated that it was related to YBMV.

Materials and methods.—Most of the plants used in this study were grown in 5- or 6-in. clay pots; some plants, e.g., tobacco, were first germinated in seedling pots and then transferred to 5- or 6-in. pots when about 2–3 in. tall. Weeds were at first dug in areas where YBMV was unknown, and were later grown from seed. Five to 10 plants of each species were inoculated with YBMV in each trial. The plants were grown in a greenhouse at 70–80°F.

Unless otherwise specified, all inoculations were made with juice extracts from systemically infected leaves of Bountiful bean or Virginia blackeye cowpea. Usually 2–3 leaves were ground in a motar and pestle with 2–3 ml of 0.1*N* sodium phosphate buffer solution at pH 7. Leaves to be inoculated were dusted with 400-mesh powdered carborundum (6). Inoculum was rubbed on with the forefinger in early tests; later, a pestle was used to minimize contamination.

Bountiful bean, cowpea, and cucumber were used as the indicator hosts and in attempts to recover YBMV from orchard weeds and from the aboveground portions of juice-inoculated plants.

On inoculated plants in the greenhouse, symptoms in winter and early spring differed from symptoms in the summer. In fact, in summer it was impossible to detect symptoms on some plants. The herbaceous host-range work was therefore carried on during cooler periods.

In interference tests with a severe and mild strain of YBMV, buds or bark chips were inserted in Lovell seedlings by the T-bud method.

Soil used in soil-vector transmission studies was collected from around the roots of YBMV-infected peach trees 6 in. below the soil surface in an orchard, Gordon Valley, Solano County. Steam-pasteurized soil used in the checks was U.C. Soil Mix II-C (2).

Results.—Of 62 plant species and varieties in 15 plant families inoculated in the host-range study, 31 species and varieties in 10 families displayed YBMV symptoms. Local-lesion symptoms appeared in 3–5 days, and systemic symptoms in 7–12 days.

Although a large number of species were infected by mechanical inoculation under greenhouse conditions, only 5—Bountiful bean, cowpea, cucumber, *Nicotiana rustica*, and *N. tabacum*—were used as differential hosts to detect YBMV in suspected plants. Bountiful bean is not an essential host, but it was found useful since it is less sensitive than cowpea to fumigation and sprays. Cucumber was always included because it was often the only host to become infected when inoculated with plant material from the field.

Diagnostic hosts.—Following is a detailed description of YBMV symptom expression in the 5 diagnostic hosts. These hosts may also be used as a quick test for YBMV in suspected trees if suitable leaf material is available.

1) *Bountiful bean.*—The first symptoms—light chlo-

rotic lesions on inoculated leaves (Fig. 1-D)—could be seen best when the leaves were shaded. The local lesions became progressively more pronounced and often became necrotic. This was followed by systemic shock on new leaves. The leaves became distorted, died, and finally dropped from the plant. Occasional plants recovered and sent out new leaves that were small and irregularly shaped.

2) *Cowpea.*—The first symptoms—light chlorotic lesions on the inoculated leaves (Fig. 2-C)—usually became necrotic later (Fig. 2-B). Systemic symptoms consisted of chlorotic lesions, vein feathering, and, later, necrotic lesions on the leaves. Then came a reddening of the leaf petioles, which finally collapsed (Fig. 2-A). The main stem sometimes collapsed below the cotyledons. If the plants were kept for an extended period, bud proliferation often took place at the cotyledonary node (Fig. 2-D). Growth from this

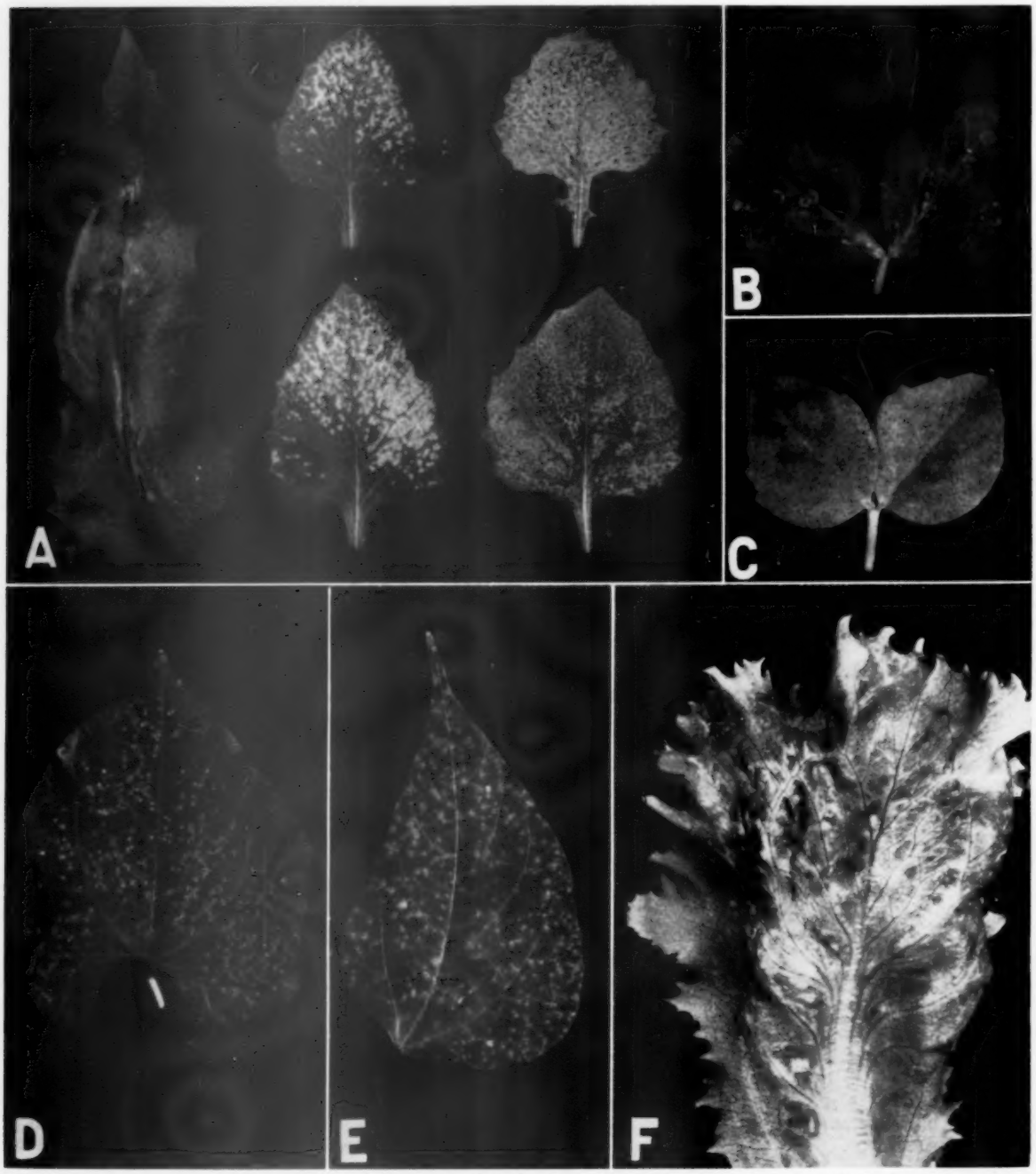


Fig. 1. Yellow bud mosaic symptoms on 4 hosts. **A)** Emilia—local chlorotic lesions and rings in leaf at left and systemic veinal chlorosis in other 4 leaves. **B,C)** Pea—local chlorotic lesions (B) and systemic veinal chlorosis (C). **D,E)** Bountiful bean—chlorotic local lesions (D) and systemic chlorotic lesions (E). **F)** Lettuce—veinal chlorosis.

point consisted of small, narrow, distorted leaves (Fig. 2-D).

3) *Cucumber.*—Local chlorotic lesions appeared on the inoculated cotyledons but did not become necrotic (Fig. 3-B). Systemic symptoms consisted of chlorotic lesions, mosaic, intraveinal chlorosis, and sharper leaf serrations. The plants usually survived and continued to grow, but were somewhat stunted.

4) *Nicotiana rustica.*—Local necrotic lesions were usually in the form of rings (Fig. 3-D). Systemic symptoms, which were necrotic rings, were occasionally as few as 1–2 per leaf. As the plants continued to grow, the symptoms appeared at the very edge of the new leaves. As the plants approached flowering, symptom expression was entirely suppressed in the new growth.

5) *Nicotiana tabacum.*—Necrotic local lesions were the only symptoms observed. They often took the shape of rings (Fig. 3-C).

Herbaceous host range and symptom expression.—Following is an account of host range and expression of symptoms. The hosts inoculated in strain differentiation work are marked with an asterisk (*).

Only local symptoms appeared on the following plant species: COMPOSITAE—*Helianthus annuus* L., sunflower. SOLANACEAE—*Datura stramonium* L.*; *Nicotiana tabacum* L.* (Fig. 3-C); and *Solanum nigrum* L., black nightshade.

Only systemic symptoms appeared on the following plant species: CARYOPHYLLACEAE.—*Stellaria media* (L.) Cyr., common chickweed. COMPOSITAE—*Lactuca sativa* L., lettuce var. Great Lakes 118 (Fig. 1-F); *Zinnia elegans* Jacq.* GERANIACEAE—*Erodium cicutarium* L'Her., red stem filaree. LEGUMINOSAE—*Glycine max* (L.) Merr., soybean; *Lupinus angustifolius* L. ROSACEAE—*Prunus persica* Batsch., peach var. Lovell (seedlings)*.

Both local and systemic symptoms appeared on the following plant species: AMARANTHACEAE—*Amaran-*

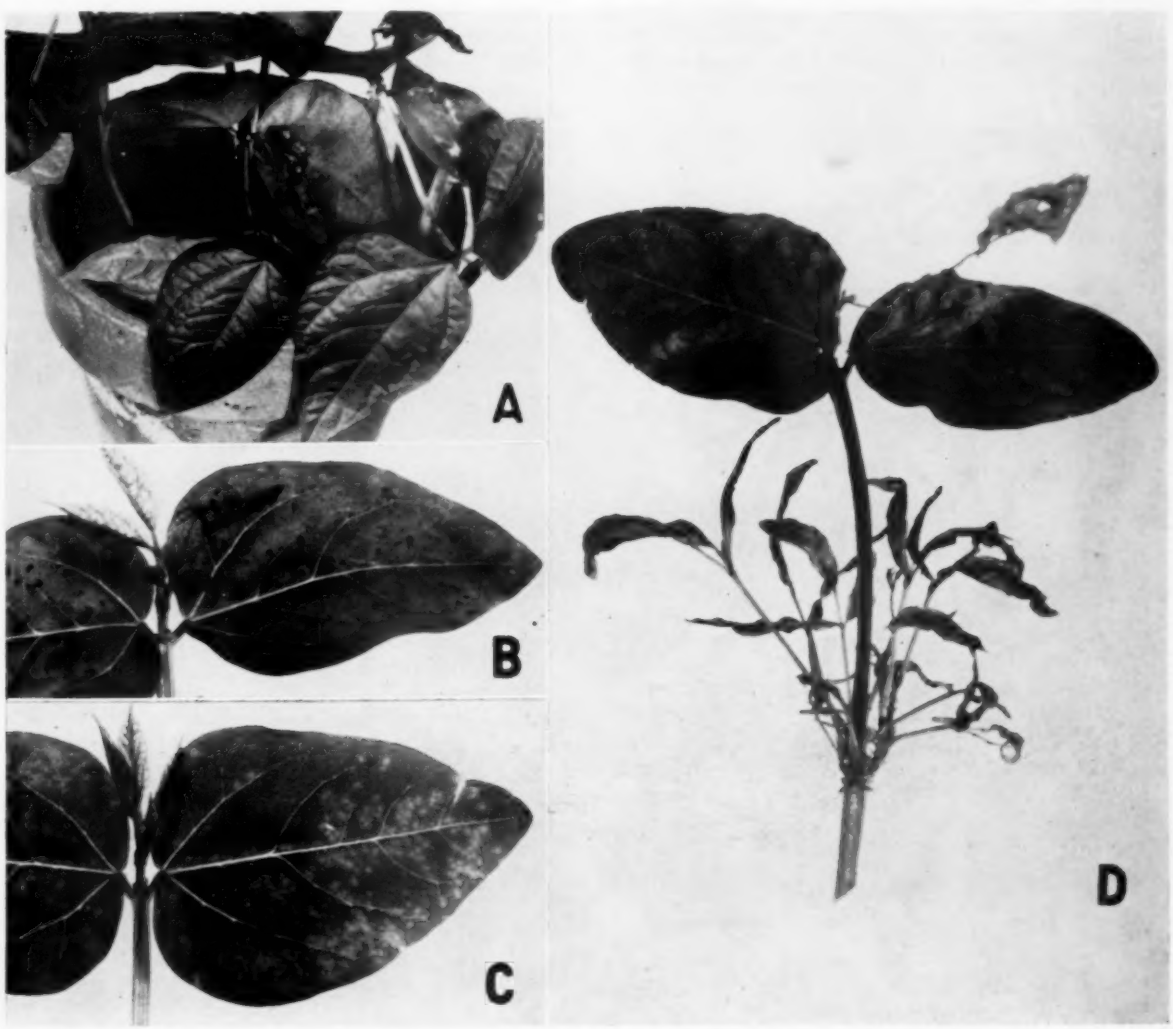


Fig. 2.—Yellow bud mosaic virus in cowpea. **A)** Local necrotic lesions on cotyledons, systemic necrotic lesions in leaves, and necrosis and collapse of stems and leaf petioles. **B)** Necrotic local lesions in cotyledons. **C)** Local chlorotic lesions in cotyledons. **D)** Distorted leaves growing from the cotyledonary node.

thus retroflexus L., rough pigweed; *Gomphrena globosa* L.* CHENOPODIACEAE—*Beta vulgaris* L., sugar beet var. S-22* (Fig. 3-A); *Chenopodium album* L., white pigweed; *C. amaranticolor* Coste & Reyn.* (Fig. 3-E); *C. capitatum* (L.) Aschers; *Spinacia oleracea* L., spinach.* COMPOSITAE—*Emilia* sp. Cass. (Fig. 1-A); *Lactuca scariola* L., prickly lettuce. CUCURBITACEAE—*Cucurbita pepo* L., pumpkin var. Small Sugar; *Cucumis sativa L.*, cucumber var. National Pickling* (Fig. 3-B). LEGUMINOSAE—*Cyamopsis psoraloides* D.C., guar; *Phaseolus vulgaris* L., bean var. Bountiful* (Fig. 1-D,E); *Pisum sativum* L., pea var. Commando #8 (Fig. 1-B,C); *Trigonella foenum-graceum* L., fenugreek; *Vigna sinensis* Savi, cowpea vars. Wisconsin blackeye, Virginia blackeye* (Fig. 2), and Black. MALVACEAE—*Gossypium hirsutum* L., cotton var. Acala 4-42. SOLANACEAE—*Nicotiana rustica* L.* (Fig. 3-D); *Petunia hybrida* Vilm.

No symptoms appeared in, nor could the virus be recovered from, the following plant species: CONVOLVULACEAE—*Convolvulus arvensis* L., orchard morning glory; *Dichondra repens* Forst.; *Ipomoea batatas* Lam., sweet potato; *I. hirsutula* Jacq. morning glory. CRUCIFERAE—*Capsella bursa-pastoris* (L.) Moench., shepherd's purse; *Brassica campestris* L., common yellow mustard. CUCURBITACEAE—*Citrullus vulgaris* Schrad., watermelon var. Klondike striped.* GRAMINEAE—*Avena sativa* L., oats var. Ventura*; *Bromus rubens* L., red brome; *Hordeum vulgare* L., barley var. Atlas 46*; *Poa annua* L.; *Triticum vulgare* Vill., wheat var. White Federation; *Zea mays* L., sweet corn var. Golden Bantam*. LEGUMINOSAE—*Medicago hispida* Gaertn., burr clover; *M. sativa* L., alfalfa vars. African, Caliverde*, and Ladak; *Melilotus indica* All., sour clover; *Trifolium pratense* L., red clover. MALVACEAE—*Malva parviflora* L., cheeseweed. ROSACEAE—*Prunus avium* L., sweet cherry var. mazzard (seedlings); *Pyrus communis* L., pear var. Winter Nelis (seedlings). SOLANACEAE—*Capsicum frutescens* L., pepper var. I. C. Wonder; *Lycopersicum esculentum* Mill., tomato var. Improved Pearson*; *Nicotiana glutinosa* L.*; *Physalis ixocarpa* Brot., ground cherry; *Solanum tuberosum* L., potato. UMBELLIFERAE—*Apium graveolens* L., celery var. Utah 10b*. VITACEAE—*Vitis vinifera* L., grape vars. Tokay and Thompson Seedless.

Virus recovery from inoculated plants.—YBMV was recovered from the above-ground portions of all plant species inoculated and displaying symptoms except: AMARANTHACEAE—*Amaranthus retroflexus* L. CARYOPHYLLACEAE—*Stellaria media* (L.) Cyr. CHENOPODIACEAE—*Beta vulgaris* L. SOLANACEAE—*Datura stra-*

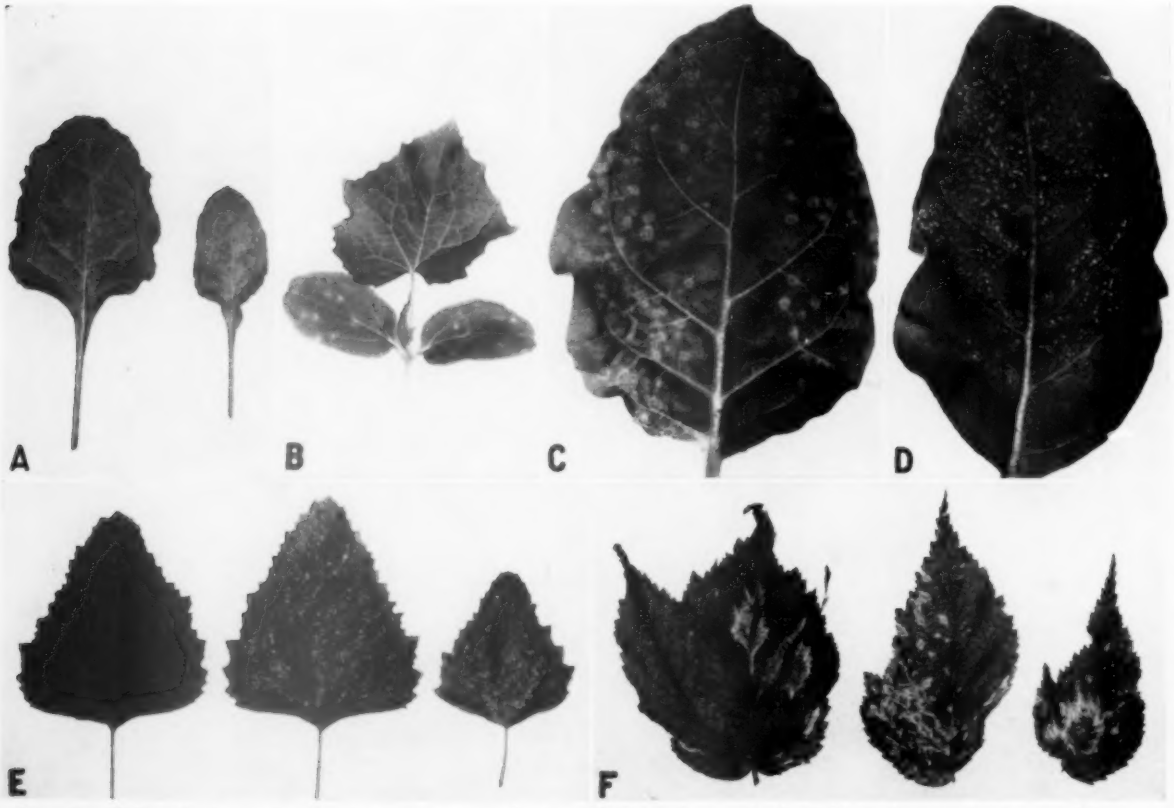


Fig. 3.—Yellow bud mosaic symptoms on various hosts. **A)** Sugar beet—check (left) and systemic rings and line pattern (right). **B)** Cucumber—local chlorotic lesions. **C)** *Nicotiana tabacum*—local necrotic lesions in the form of rings. **D)** *N. rustica*—necrotic local lesions and etched rings. **E)** *Chenopodium amaranticolor*—check leaf on the left, local chlorotic lesions on center leaf, and systemic chlorotic lesions on leaf at the right. **F)** Raspberry—vein feathering and mosaic symptoms in leaflets from naturally infected plant.

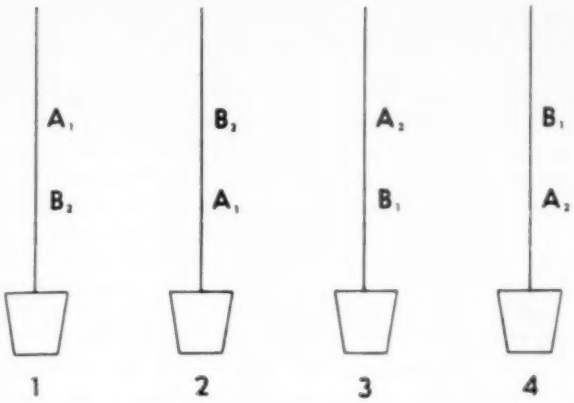


Fig. 4.—Interference tests between 2 strains of YBMV on 4 groups of Lovell peach seedlings, 8 trees per group. A = YBMV from peach. B = Himalaya blackberry mosaic virus. A_1 and B_1 inoculations made June 30, 1958. A_2 and B_2 inoculations made September 17, 1958. Inoculation sites of A and B were 4 and 8 in. above the soil line.

monium L. Virus was recovered from the roots of *Stellaria media*.

Isolation attempts from weeds from the field.—Weeds growing under YBMV-infected peach trees were collected for virus assay at various times in March and April of 1957 and 1958, and, if still available, in October, 1957: *Amsinckia Douglasiana* DC., *Avena fatua* L., *Bromus rubens* L., *Calandrinia caulescens* var. *Menziesii* Gray, *Convolvulus arvensis* L., *Erodium cicutarius* L'Her, *Lactuca scariola* L., *Malva parviflora* L., *Medicago hispida* Gaertn., *Montia perfoliata* (Donn) Howell, *Raphanus sativus* L., *Sonchus oleraceus* L., and *Stellaria media* (L.) Cyr. As much of the root system as possible was retained, the soil was washed from the roots in running water, and isolations were attempted from both the roots and the aboveground portions. The only success was from the roots of a plant of *Malva parviflora* obtained in October, 1957, from an orchard in Gordon Valley, Solano County.

Reactions with the above strain in test hosts were so mild that its identity was not certain at first. After several transfers through cowpea, however, it produced symptoms that were essentially similar to those from YBMV from peach, though slower in developing. This is believed to be the first successful recovery of YBMV from a naturally infected orchard weed. A more intensive assay of weeds in orchards would probably reveal additional specimens infected with YBMV.

Strains of YBMV.—To determine whether strains of YBMV exist, 20 herbaceous species were mechanically inoculated with the four original isolates—from: 1) raspberry (Fig. 3-F) taken from a planting in Suisun Valley, Solano County; 2) soil that had transmitted YBMV to peach in the greenhouse; 3) Himalaya blackberry from Santa Cruz County; and 4) *Malva parviflora* from an orchard in Gordon Valley, Solano County. The varieties tested were those indicated by an asterisk (*) in the section on host range.

The raspberry isolate was taken from a naturally infected raspberry plant growing on land previously occupied by peach trees known to be infected with YBMV.

From symptom expression, it is believed that the isolates from raspberry, blackberry, and Malva are all strains of YBMV. The 4 strains, differing little

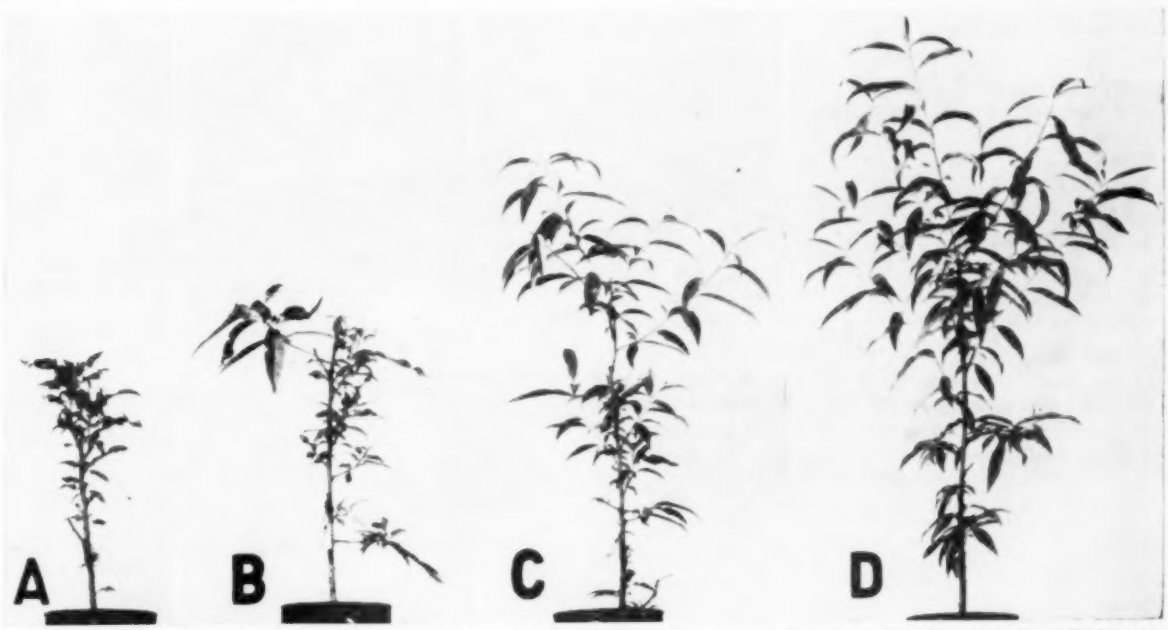


Fig. 5.—Lovell seedlings. Cross-protection between YBMV from peach and Himalaya blackberry. **A)** YBMV from peach (inoculated September 17, 1958). **B)** Inoculated June 30, 1958, with YBMV from peach, and September 17, 1958, with Himalaya blackberry mosaic virus. **C)** Inoculated June 30, 1958, with Himalaya blackberry isolate, and September 17, 1958, with the peach isolate. **D)** Inoculated September 17, 1958, with Himalaya blackberry isolate.

except in severity of symptoms, were classified in descending order of severity as follows: raspberry, peach, Himalaya blackberry, and *Malva parviflora*. Severity of disease was measured by speed of disease development. The only difference noted besides severity was that the Malva strain was not transmitted mechanically to Lovell seedlings in a limited number of tests.

Interference of virus strains in peach.—To determine whether a mild strain (Himalaya blackberry) would protect against a more severe strain (peach), Lovell peach seedlings grown in the greenhouse from seed and selected for uniformity were divided into 4 lots of 8 each and inoculated with buds or bark chips inserted in the stems at the places indicated in Fig. 4. Check seedlings received the strains separately.

When the severe strain was budded before the mild strain, either above or below (Fig. 5-B), the symptoms matched those of peach seedlings inoculated with the severe strain alone (Fig. 5-A). Interference was definite, however, when the mild strain was budded before the severe strain, either above or below (Fig. 5-C), though budding the severe strain below gave slightly greater interference. Whether the interference will continue in these trees is not yet known.

Seed transmission.—In an experiment designed to determine whether YBMV is seed-borne in herbaceous plants, juice-inoculated Bountiful bean and Zinnia plants were grown until seed was set. Seeds harvested from these infected plants were planted in steamed soil on December 20, 1956. No virus symptoms were observed, nor could the virus be recovered from any of the plants 1–2 months after the seeds were planted. All the plants were discarded after 3 months. Further work is required since this phase of work was limited.

In tests in 1956 and 1957, plants produced from clean seed of Bountiful bean, cowpea, or cucumber were not infected with YBMV by being grown in steamed soil in which juice-inoculated Bountiful bean plants had grown. Apparently no vector was present in the steamed soil.

Site of natural infection.—On March 29, 1957, unsteamed soil from around the roots of YBMV-infected peach trees was used to determine whether a vector must leave the soil and make its way to the leaves or stems in order to transmit YBMV. Small Lovell seedlings were planted in the soil in 100 1-qt Mason jars. A portion of the lid was cut out so the seedling could grow through the top. The openings around the stems were sealed with glass wool in 50 lids and left open in 50. The two groups of seedlings were kept in separate greenhouses. Checks were 50 Lovell seedlings in jars filled with steamed soil, 25 with sealed tops and 25 left open. Equal numbers of the sealed and unsealed checks were interspersed among the open and sealed jars containing the unsteamed soil.

During March and April, 1958, 9 seedlings in open jars and 8 in sealed jars filled with unsteamed soil developed symptoms suggesting YBMV. The virus was isolated from the leaves of all such seedlings. No symptoms appeared in, nor could the virus be isolated from, any of the checks. From these limited tests, it is suggested that infection probably took place through the roots or the portion of the stem near the soil. These results are in line with recent work suggesting that the soil vector of YBMV is a nematode (3).—Department of Plant Pathology, University of California, Davis.

LITERATURE CITED

1. Alcorn, S., S. Wilhelm, and H. E. Thomas. 1955. A mosaic disease of Himalaya blackberry. Phytopathology 45:272-274.

2. Baker, K. F., ed. 1957. The U. C. system of producing healthy container-grown plants. University of California, Division of Agricultural Sciences, Manual 23. 332 p.

3. Breece, J. R., and W. H. Hart. 1959. A possible association of nematodes with the spread of peach yellow bud mosaic. Plant Disease Reptr. 43:989-990.

4. Karle, H. P. 1957. Mechanical transmission of yellow bud mosaic virus. (Abstr.) Phytopathology 47:526.

5. Karle, H. P., and G. Nyland. 1959. Yellow bud mosaic in almond. Plant Disease Reptr. 43:520-521.

6. Rawlins, T. E., and C. M. Tompkins. 1936. Studies on the effect of carborundum as an abrasive in plant virus inoculation. Phytopathology 26:578-587.

7. Schlocker, A., R. L. McClain, and J. P. Hill. 1958. Peach yellow bud mosaic. Calif. Dept. of Agr. Bull. 47(2):183-184.

8. Thomas, H. E., C. E. Scott, E. E. Wilson, and J. H. Freitag. 1944. Dissemination of a peach mosaic. Phytopathology 34:658-661.

9. Thomas, H. E., and T. E. Rawlins. 1939. Some mosaic diseases of Prunus species. Investigations on Winters peach mosaic. Hilgardia 12:623-644.

10. Thomas, H. E., and T. E. Rawlins. 1951. Yellow bud mosaic. U. S. Dept. Agr., Agr. Handbook 10, Virus diseases and other disorders with virus-like symptoms on stone fruits in North America, p. 53-55.

11. Wagnon, H. K., and A. Schlocker. 1953. Peach yellow bud mosaic. Calif. Dept. Agr. Bull. 42:266-267.

12. Wagnon, H. K., and J. R. Breece. 1955. Evidence of retention of peach yellow bud mosaic virus in soil. Phytopathology 45:696.

13. Yarwood, C. E. 1953. Quick virus inoculation by rubbing with fresh leaf discs. Plant Disease Reptr. 37:501-502.

14. Yarwood, C. E., and H. E. Thomas. 1954. Mechanical transmission of three fruit tree viruses. (Abstr.) Phytopathology 44:511.

15. Yarwood, C. E. 1956. Mechanical transmission of peach yellow bud mosaic virus. Plant Disease Reptr. 40:299.

16. Yarwood, C. E. 1957. Juice transmission of viruses to peach. (Abstr.) Phytopathology 47:38.

RELATIONS OF VARIETY, TEMPERATURE, AND SEED IMMATURITY TO PRE-EMERGENCE DAMPING-OFF OF CASTORBEAN

C. A. Thomas

Eight years of observations on field stands of castorbeans showed differences in stand apparently due to differences in the quality of commercial seed lots. The differences were not evident in normal methods of determining seed quality, such as bushel weight, weight per 200 seed, seed appearance, or germination under optimum conditions. Studies developed a "cold test" that proved satisfactory. Discussed herein are the effects of some factors on pre-emergence damping-off by *Pythium* spp., noted in developing the cold test.

The effects of variety and temperature on pre-emergence damping-off were studied simultaneously. Seeds were harvested from all racemes of 18 varieties of castorbeans grown in field plots at Beltsville, Maryland. Twenty seeds of a single variety were planted in composted soil mixed 1:1 with vermiculite in each of 32 one-quart cans. After being watered to field capacity, the cans were covered with burlap to reduce moisture loss. Eight cans of each lot were placed in each of 4 chambers with constant temperatures of 30, 20, 15, and 10°C. Stands in the 30° series were counted after 2 weeks. Cans at the other temperatures were moved to 30° after 2 weeks, and stands were counted 2 weeks later. The tests were repeated 2 or more times.

The effect of seed immaturity on pre-emergence damping-off was studied with seeds harvested separately from the first, second, and third sequential sets of racemes of the varieties Cimarron and Dawn.

Table 1.—Average percentage stands obtained with castorbean seed of different varieties in moist composted soil held 14 days at 4 temperatures.

Variety	Holding temperature for first 14 days (°C)			
	30	20[a]	15[a]	10[a]
Cimarron	76.2	16.8	81.5	79.4
Baker 43	65.0	41.5	85.0	85.0
Illinois 224A	81.5	45.0	70.4	71.8
Baker 195	81.8	38.5	83.3	88.7
U. S. 39	85.0	50.1	35.5	21.6
U. S. 65	76.5	56.5	23.0	15.2
Baker 296	90.0	41.9	28.0	13.6
Nebraska 145-4	80.5	67.8	61.0	51.7
Custer	69.2	64.5	62.2	56.7
U. S. 74	89.0	78.1	46.8	30.6
U. S. 101	66.3	48.0	23.2	15.1
Dawn	50.9	27.6	13.5	10.8
Illinois 48-36	63.0	15.4	10.7	0.0
Cimarron Hybrid[b]	93.4	26.3	63.1	50.8
Baker 195 Hybrid[b]	81.5	28.5	45.0	35.3
3/415-9 Hybrid[b]	91.5	25.0	50.4	33.6
U. S. 74 Hybrid[b]	79.8	31.7	55.5	42.8
U. S. 101 Hybrid[b]	72.2	25.7	50.0	38.3

[a] Subsequently held 14 days at 30°.
[b] Nebraska 145.4 was the female parent.

Tests were run at 20 and 10°. Other procedures were the same as described above.

Isolations from seeds that rotted without germinating yielded *Pythium* spp., mainly *P. ultimum* Trow, more frequently than all other fungi combined. Seed rot and stand loss were severe in steamed soil artificially infested with isolates of *P. ultimum*.

Germination under optimum conditions was determined by planting 2 100-seed samples of each seed lot in steamed soil in flats held at 30°. Germination of each lot was over 90%.

There were marked differences among the varieties (Table 1) in temperature that gave maximum stand loss—10° for most inbred varieties; 20° for Cimarron, Illinois 224A, Baker 43, and Baker 195, however. Hybrid varieties had poorer stands from 20° and 10° than from 30° or 15°.

Seeds of Cimarron and Dawn from the first, second, and third sequential sets of racemes, presumed to differ in maturity, differed markedly in the stands produced (Table 2). The temperature treatment that produced maximum loss of stand differed with the varieties and appeared unaffected by seed maturity.

The experiments showed that the incubation temperature used in a "cold test" for castorbeans should not be the same for all varieties. The test would thereby differ from cold tests for corn (1). The lack of appreciable seed rot in varieties like Cimarron in the 10° and 15° treatments appeared to be due to dormancy. In blotter germination tests seeds of those varieties showed no signs of germination after 21 days at 15°. Seeds of the other varieties at 15° germinated in 14 days.

Immaturity, due to early defoliation by drought, frost, chemical desiccants, or disease, has appeared to be the chief factor responsible for the predisposition of castorbean seed to pre-emergence damping-off. Recent studies have shown that this predisposition is correlated with seed permeability and can be accurately determined from the conductivity and reducing-sugar content of seed steep water (2, 3).

Seed treatment is known to be fairly effective in

Table 2.—Average percentage stands obtained with castorbean seed from the first, second, and third sequential racemes of 2 varieties in moist composted soil held 14 days at 2 temperatures and subsequently held 14 days at 30°C.

Variety and raceme sequence	Holding temperature for first 14 days (°C)	
	10	20
Cimarron:		
1	90.6	36.4
2	89.0	29.1
3	91.4	11.0
Dawn:		
1	39.8	78.7
2	27.2	57.5
3	5.3	44.0

controlling pre-emergence damping-off of castorbean. The use of a "cold test" or seed-permeability measurements, however, is desirable, because seed so selected produces more vigorous seedlings.—Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

LITERATURE CITED

1. Hoppe, P. E. 1953. Infections of corn seedlings. *In* Plant Diseases. Yearbook Agr. U. S. Dept. Agr. 1953:377-380.
2. Presley, J. T. 1958. Relations of protoplast permeability to cotton seed viability and predisposition to seedling disease. Plant Disease Reptr. 42:852.
3. Thomas, C. A. Permeability measurements of castorbean seed indicative of cold test performance. (In preparation).

A WHOLE-MOUNT TECHNIQUE FOR STUDYING INFECTED LEAVES

P. K. Isaac

In studying the extent and distribution of fungal hyphae infecting plant tissue, differential staining and searching serial sections by standard procedures may take much time. Popp (2) recently demonstrated the great advantage of a whole-mount technique in the study of smut-infected embryos. This paper describes a method whereby a clearing fixative is followed by a staining mountant to give permanent mounts that are transparent enough for use with such material as whole portions of leaves, thick sections, or small roots or stems.

The infected leaves are cut into portions about 5 mm square and dropped into a 95:5 fixative mixture of dioxan and propionic acid (reagent grade).

To avoid diluting the fixative, and to ensure complete extraction, about 5 ml of fixative should be allowed for each portion of leaf. The flask containing the fixative is corked and placed in a paraffin embedding oven at 60°C until the leaves are colorless. The time taken to complete this stage varies with the material, but is usually less than 24 hours. Then the specimens are removed from the fixative and washed in distilled water for at least 3 hours. The specimens are then placed on clean microscope slides, a few drops of Haem-gum staining mountant (1) are added, and a coverslip is lowered in place. The correct quantity of mountant to use will vary with the thickness of the specimen, but enough should be used to allow a ring of excess mountant to form around the edge of the coverslip, which will allow for the slight shrinkage of mountant that occurs on drying.

Accumulation of the haematoxylin stain can be seen to start in the nuclei a few minutes after the slides are prepared, but at this stage the transparency of the tissue is poor owing to the wide range of refractive indices in the preparation. This situation improves as the mounting medium penetrates the specimen, and clearing is complete in most cases within a few days or a week. The *Erysiphe* oidia shown in Fig. 1-C were on the underside of the mount and photographed through the thickness of the leaf, which shows the degree of optical homogeneity achieved. In such cases

Fig. 1.—Photomicrographs of infected leaves cleared in a dioxan fixative and mounted in Haem-gum staining mountant. **A)** Whole-leaf mount showing dikaryotic cells in a developing uredial initial of *Melampsora lini* (Pers) Lév. (×1200). **B)** Portion of a wheat leaf infected with *Puccinia graminis* Pers. f.s. *tritici* Erikss. & E. Henn. teased out before mounting, showing a dikaryotic pair of nuclei in a vegetative hypha and a haustorium developing close to a host nucleus (×400). **C)** Whole mount of a wheat leaf with oidia of *Erysiphe graminis* DC. on the underside to demonstrate the optical homogeneity of the preparation (×300).

it is necessary to adjust the tube length of the microscope to overcome the aplanatic error introduced by the thickness of the mount. An optical tube length corrector (W. Watson & Sons, 313 High Holborn, London, W.C.1, England) is the most convenient method of achieving this, for the degree of correction required by thick specimens is frequently beyond the scope of the correction collars fitted to many 4-mm objectives.

The preparations show considerable cytological detail (Fig. 1-A,C) and differentiation between host and parasite is obtained by the differences in intensity of the stain rather than by any color differences; the pathogen stains more deeply than the cytoplasm of the host.

The procedure was developed and first used for studying invasion by cereal rusts, but has since been used with success for many other fungal pathogens as well as bacteria and nematodes that invade leaves or seedlings. It has also proved reliable enough for use in plant pathology classes, where seeing the whole tissue in 3 dimensions helps the student to better understanding of the relations between host and pathogen. This is particularly true with a succession of invading organisms, as in many cases of damping-off of seedlings.—Department of Botany, University of Manitoba, Winnipeg, Manitoba, Canada.

LITERATURE CITED

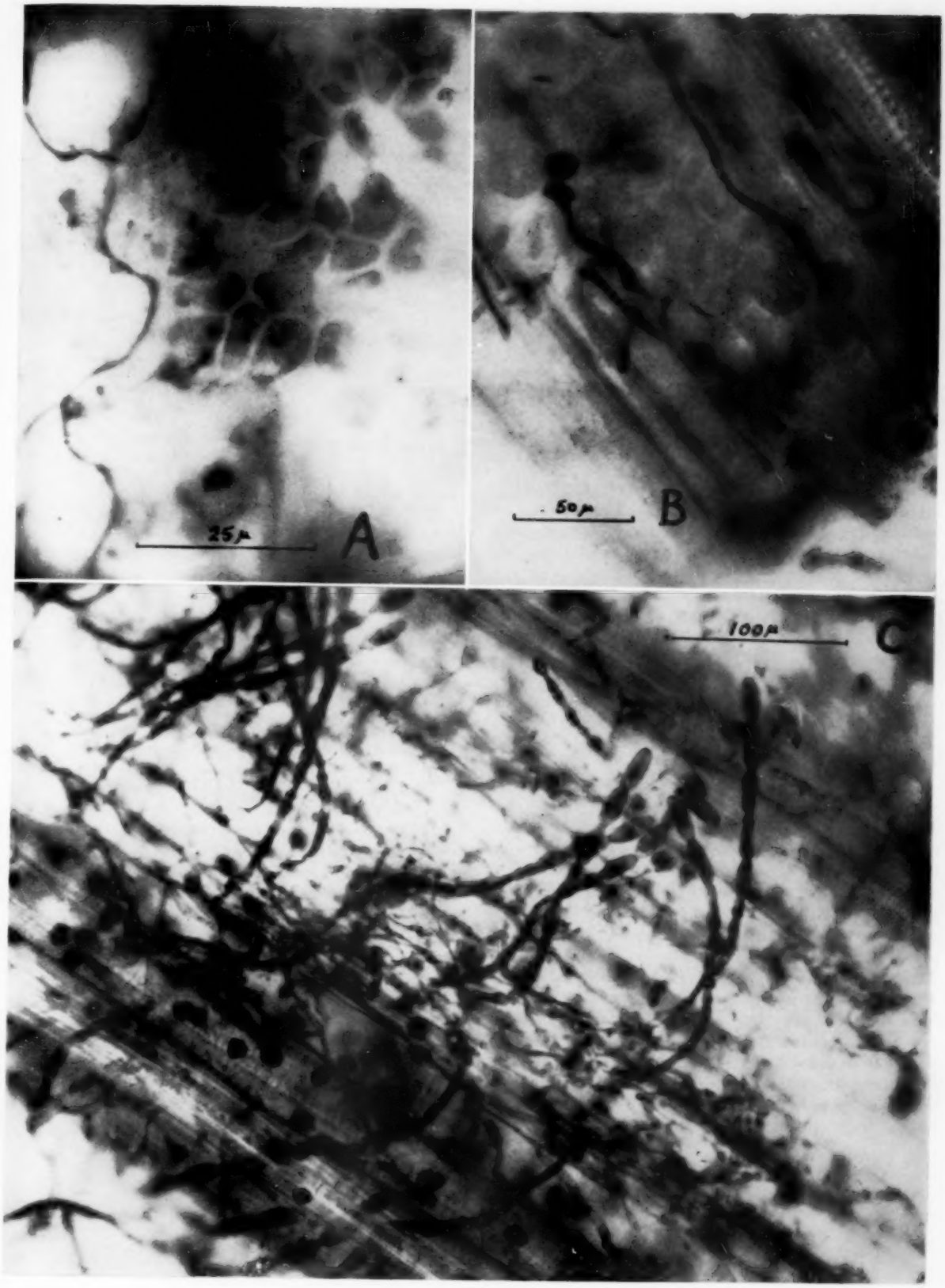
1. Isaac, P. K. 1958. A hematoxylin staining mountant for micro-organisms. Stain Technol. 33:261-264 p.
2. Popp, W. 1958. An improved method of detecting loose-smut mycelium in whole embryos of wheat and barley. Phytopathology 48:641-644 p.

GAMMA RADIATION DOSE RESPONSE OF SOME DECAY PATHOGENS

Louis Beraha, M. A. Smith, and W. R. Wright

Respectively, Pathologist, Senior Pathologist, and Associate Pathologist, Market Quality Research Division, Agricultural Marketing Service, U.S.D.A., Chicago, Illinois.

Investigation conducted by the Market Quality Research Division, Agricultural Marketing Service, U.S.D.A. This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number 1054 in the series of papers approved for publication. The views or conclusions contained herein are those of the authors, and not to be construed as necessarily reflecting the views or endorsement of the Department of Defense. Most of the work was done under the supervision of G. B. Ramsey, formerly Principal Pathologist at the Chicago Laboratory.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C

Table 1.—Approximate doses of gamma radiation lethal to young actively growing mycelium of some plant pathogens.

Organism	Source	Dose (×100,000 rad) on		
		Tochinai	Czapek	Host
Phytophthora infestans (Mont.) d By.	potato	0.25	0.25	0.25
Phomopsis citri Fawc.	orange	0.44–0.96	0.44–0.96	0.91–1.15
Penicillium digitatum Sacc.	lemon	1.1 –1.48	0.44–0.94	1.82–2.10
P. italicum Wehmer	orange	1.43–1.47	1.19–1.43	1.57–1.82
P. expansum (Lk.) Thom	apple	1.35–1.40	1.95–2.52	1.82–2.74
Pythium debaryanum Hesse	potato	1.82–2.28	0.69–1.37	1.57–1.82
Botrytis cinerea Pers. ex Fr.	grape and strawberry	0.95–1.86	0.95–1.86	2.74–4.56
B. cinerea	blueberry	1.54–2.03	1.54–2.03	
B. allii Munn	onion	1.33–1.83	1.33–1.83	2.74–3.65
Phoma sp.	blueberry	1.54–2.03	0.5 –0.97	
Monilinia fructicola (Wint.) Honey	peach	1.38–1.85	0.90–1.38	1.37–1.82
Sclerotinia sclerotiorum (Lib.) d By.	bean	1.73–2.13	1.73–2.13	2.28–2.73
Pellicularia rolfsii (Curzi) E. West	watermelon	2.43–2.56	1.54–2.0	
Cladosporium sp.	lemon	2.56–4.27	2.56–4.27	4.56–9.12
Gloeosporium musarum Cke. & Mass.	banana	2.41–2.92	2.41–2.92	
Gloeosporium sp.	blueberry	2.98–4.85	2.98–4.85	
Rhizopus nigricans Ehrenb. ex Fr.	peach	2.74–3.52	3.52–4.43	1.82–2.28
R. nigricans	sweet potato	2.74–3.52	3.52–4.43	4.56–9.12
Stemphylium radicina (Meier, Dresch. & Eddy) Neerg.	carrot	2.66–3.61	2.66–3.61	3.65–4.56
Alternaria tenuis Auct.	tomato	4.2 –4.57	4.2 –4.57	2.74–4.56
A. citri Ell. & Pierce	lemon	4.27–4.56	4.27–4.56	2.74–4.56
Diplodia natalensis P. Evans	peach	4.65–8.91	2.56–4.65	2.74–4.56

During experiments on extending the shelf life of fruits and vegetables through gamma irradiation we tested *in vitro* and *in vivo* some 21 fungal agents of decay. Of particular interest was variation of the species in sensitivity to radiation. Dissimilarity in response served as a guide to the range of effective doses for our work. Studies employing some of these data for decay control at different storage temperatures have been reported (1, 2).

Conidia of the fungi tested were removed from well-sporulating cultures, suspended in sterile distilled water, and uniformly streaked onto either Czapek's or Tochinai's agar slants in small tubes. In determining sensitivity of the organisms in the host, the commodities tested were inoculated by dipping injured surface tissue into a suspension of conidia. For each dose tested in each of two trials, 6 tubes of each medium were used as were 8 artificially inoculated host samples. After 18–20 hours at 75–80°F, sufficient for germination and formation of young hyphae, the culture tubes and hosts were placed in No. 2 cans and irradiated at the High Level Gamma Irradiation Facility at the Argonne National Laboratory, Lemont, Illinois. Irradiation was performed at a rate of 1–2 million rads per hour over a dose range of 25,000–1,000,000 rads (a rad unit corresponds to the amount of ionizing radiation that would deliver 100 ergs to 1 g of wet tissue). Following irradiation, cultures were observed for resumption of growth either *in situ* or from irradiated fungal fragments transferred to potato-dextrose agar. In the host, growth was determined by development of decay or by microscopic tissue examination. Of the doses tested, the highest dose at which some growth resumed and the lowest dose that did not result in any growth, formed the limits of the approximate lethal dose range.

The approximate lethal doses *in vitro* of gamma radiation (Table 1) indicate extreme sensitivity of *Phytophthora infestans* and *Phomopsis citri;* variability in species of fungi of the same genus, and in general more resistance by *Cladosporium* sp., *Gloeosporium* spp., *Rhizopus nigricans, Stemphylium radicina, Alternaria* spp., and *Diplodia natalensis.* In general, grouping the fungi tested into classes of very sensitive, moderately resistant and resistant would not always show the same relative sensitivity in the host as in the 2 culture media. The difficulty in obtaining, for the most part, a uniform lethal dose on 3 different media, demonstrates the influence of the substrate on survival. —Agricultural Marketing Service, U. S. Department of Agriculture, Chicago, Illinois.

LITERATURE CITED

1. Beraha, L., G. B. Ramsey, M. A. Smith, and W. R. Wright. 1959. Factors influencing the use of gamma radiation to control decay of lemons and oranges. Phytopathology 49:91-96.

2. Beraha, L., G. B. Ramsey, M. A. Smith, and W. R. Wright. 1959. Effects of gamma radiation on brown rot and Rhizopus rot of peaches and the causal organisms. Phytopathology 49:354-356.

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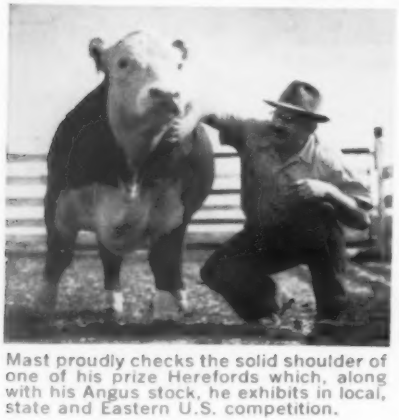

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NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

The 8th Annual Meeting and Conference of the Canadian Agricultural Chemicals Association will be held at the Britannia Hotel, Lake of Bays, Muskoka, Ontario, on September 12-14. Addresses will include "Evaluating New Pesticides" by Dr. F. Glen, Director General, Research Branch, Canada Department of Agriculture; "Insect Resistance" by Dr. A. W. A. Brown, Department of Entomology, Western University, London, Ontario; "Tariff Reference 120" by Mr. J. A. Davis, Chairman, Chemical Industry Tariff Committee; "Selling and Advertising" by Mr. Louis F. Czufin, California Spray Chemical Corp.

Plant pathologists K. W. Kreitlow and W. H. Sill, Jr., have been designated official representatives of the Society to the 8th International Grassland Congress, to be held in Reading, England, July 11-21.

Forest pathologists Bratislav Zak and Craig W. Bryan, with the U. S. Forest Service, have recently joined the staff of the Department of Plant Pathology and Plant Breeding at the University of Georgia in Athens. They previously had been affiliated with the University of Georgia Forestry School.

Plant pathologist William A. Haglund has assumed his new duties as Assistant Plant Pathologist at the Northwest Washington Experiment Station at Mt. Vernon, Washington. Dr. Haglund's duties are concerned with diseases of vegetables and small fruits, with special emphasis on the nature and control of the root rot of strawberry, peas, and other vegetable crops.

Canadian graduate students in plant pathology at Universities in the United States are asked to contact Dr. A. J. Skolko, Associate Director of Program, Research Branch, Canada Department of Agriculture, Ottawa, Ontario, Canada.

Phytopathologist John P. Jones, formerly with the USDA and located at Stoneville, Mississippi, has recently joined the staff of the Department of Plant Pathology, University of Arkansas, Fayetteville. Dr. Jones will be engaged in research on diseases of small grains.

After 39 years' service Professor J. M. Raeder retired June 30 from the staffs of the University of Idaho and the Idaho Agricultural Experiment Station. During his tenure he taught the introductory course in Plant Pathology at the University and conducted investigational work on the diseases of potatoes, grain and beans.

Phytopathologist J. G. Leach, having reached the age of 65 and in accordance with University policy, on June 30 relinquished administrative duties as Head of the Department of Plant Pathology, Bacteriology and Entomology at West Virginia University. He will continue as Professor of Plant Pathology. Dr. Leach will be succeeded as Department Head by H. L. Barnett, Professor of Mycology, in the same Department.

The "Results of 1959 Fungicide Tests" has again been printed and is available at $1.00 per single copy. Copies can be secured only from Dr. A. B. Groves, Department of Plant Pathology and Physiology, Winchester Fruit Research Laboratory, Rural Route 3, Winchester, Virginia. All orders should be accompanied by remittances made out to The American Phytopathological Society. An added charge will be made for postage and handling where orders must be billed.

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